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Study of Fungicidal Activity of Synthesized Bio Active 4, 4'-Dihydroxy Bibenzyl Incorporating Various Heterocyclic Moieties

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Abstract-Bibenzyl are naturally occurring potential fungicidesThey have low toxicity to mammal and higher plants because they are biodegraded into primary metabolites viz.Phenylene and acetic acid.. It has been found that natural as well as synthetic bibenzyls both show antifungal activity. Inspite of existing large number of conventional fungicides incorporating heterocycles, only few reports are available, in literature on those incorporating bibenzyl With the hope of further exploring new antifungal bibenzyls having heterocyclic moieties it was considered to synthesize new potentially bio-active, safe, environment friendly fungicides with aim to increase permeability into the fungal cell. Several 4,4,-dihydroxybibenzyl incorporating heterocyclic moieties have been designed in such a way so that most of them incorporate toxophorically important grouping for fungi. These compounds have been screened for their fungicidal activity against two fungal species, viz, Aspergillus niger and Fusarium oxysporium. A possible relationship between screened results and structural features of the tested compound has been deduced and further fungicidal activities of various bibenzyl incorporating heterocyclic moieties have been compared. Most of the synthesized compounds inhibited 50% growth of the fungus and some compounds inhibited 80-90% growth of the fungus.

Index Terms— Biodegraded, Bio-Active, 4, 4'-Dihydroxy Bibenzyl, **Fungicidal Activity**.

I. INTRODUCTION

Fungi constitute a large group of non-vascular and achlorophyllous plants which, because of the lack of chlorophyll are unable to synthesize their own food thus they are either saprophyts, obtaining their food from dead organic matter or parasites obtaining food from the bodies of living plants and animals. Fungi are greatest enemy of man kind because they are responsible for a great loss to our food supplies both in fields and stores.

Fungicides are considered core chemicals that kill the fungus spores and mycelium. Presently fungicides play an important role to boost up the production of agricultural crops, industrial production, prolonging the utility of manufactured products and controlling the various human and fungal diseases. In view of the aforesaid acts of fungi, one may very well understand the importance of fungi toxic chemicals in agriculture as well as in industry. The fungi toxic chemicals may exhibit:

(1)**Fungistatic action**: which implies a continuous interference as long as the organism is in contact of toxicant.

(2)**Fungicidal action**: which denotes a persistent action even often withdrawal of toxicant

Literature survey on bryophytes have shown that bryophytes as well as extract of the chemical constituent of bryophytes are not damaged by fungi. The reason why bryoppytes are not affected by fungus is the presence of structural variants of bibenzyls viz.lunularic acid and lunularin, and bis bibenzyl viz plagiochin,marchatia etc[1]It has been found that natural as well as synthetic bibenzyls both show antifungal activity[2]**,**4"-thiazolidinon[3-5],Triazole[6-7]1,3,4-Oxadiazoles [8-9]thiazole[10],and thiadiazoles[11]are known to posses various biological activities. Prompted by these observation we undertook the synthesis of heterocyclic compound in which 4"-thiazolidinone, triazole, oxadiazole, thiazoles, and thiadiazoles moieties are present. When these moieties are attached with bibenzyl, which itself is antifungal, its activity increases many folds. The various synthesized compound have been screened for their antifungal activity against two fungal species, viz, Aspergillus Niger and Fusarium oxysporium.A commercial fungicide Dithane M-45 was also tested under similar conditions for comparing the results.

II. METHOD AND MATERIALS

Bibenzyl incorporating heterocyclic moieties like 4"-thiazolidinon[triazole,1,3,4-Oxadiazoles,thiazoles, and thiadiazoleshave been synthesized experimentally[12-14].The synthesized compounds have been screened for

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their fungicidal activity against two fungal species viz, , Aspergillus niger and Fusarium oxysporium by using following Agar Plate technique[15].

Organism: One week old cultures of Aspergillus niger and Fusarium oxysporium were used.

Medium: The following Potato Dextrose Agar (PDA) medium has been used.

1. Potato infusion-200g
2. Dextrose-20g
3. Agar-15g

Distilled Water-1litre

The antifungal activity of each compound was evaluated at three different concentrations viz,1000ppm,100ppm and 10 ppm.The compounds were tested either as solution or suspension in acetone: water mixture.PDA medium shaken well and sterilized it. The culture medium was taken into pre-sterlized petriplates.A fungal disc of 5mm diameter, cut out with the help of sterilized cork borer from the periphery of one week old culture of test fungus already planted on PDA medium, was inoculated in the centre of each petriplates in an inverted position to bring the mycelia in direct contact with the medium. The number of replicate assays in each case three whereas, six replications of the controls were provided. The plates were incubated at 20^0C for 96hrs.No.remarkable morphpological change was observed in the developing fungi. A commercial fungicide, Dithane M-45(a mixed manganous and zinc salt of N, N, ethylenebisdithiocarbamic acid) was also tested under similar conditions for comparing the results.

Expression of Percent Inhibition:

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

$\%inhibition=100\times(C-T)/C$

Where,

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

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

The antifungal activity displayed by various groups of compounds is recorded in table1-4.For the highly active compound, it was ascertained whether these were fungistaticor fungicidal. Thus, following the procedure of poison food method the compounds were added separately to PDA medium in different petriplates to maintain the final concentrations at their respective lethal doses. The test fungi were inoculated in the centre of these petriplates and incubated at 28^0C for 96 hr.

After which the percent inhibition of the mycelia growth compared with that in control dishes, washed with sterilized distilled water and reinoculated in fresh petridishes containing only the PDA medium. The plates were incubated for 96 hrs. at 28^0C and percent inhibition recorded.

III. RESULTS AND DISCUSSION

First series:

4,4'-bis[N-(2"-aryl-5"-methyl/unsubstituted-4"-thiazolidinon-3"-yl)acetamidoxy]bibenzyls

All the synthesized compounds **Ia-f and 1Ia-l (fig1)** were screened against A.niger and F.oxysporium for their antifungal activity and the screening data are recorded in **Table1.**It is clear from the antifungal screening data that all the screened compounds inhibited 49-100% mycelia growth both test fungi at 1000ppmconc,but their activity decreased at their activity decreased at lower conc.(100,10ppm).The most active of these **IIc,IIe** and **IIf** have displayed fungicidal action equivalent to that of the commercial fungicide Dithjane M-45 at 1000ppm conc. The compound **Ia-f** are less active than their successors **IIa-l**.This has demonstrated that introduction of thiazolidinone nucleus plays a key role in the fungi toxicity of these compounds.However,the compound **IIc,IIe** and **IIf,IIh,IIi,IIk,IIl** were most active. It was however noted that the introduction of NO_2 group into the phenyl nucleus of these compounds augmented the fungicidal action appreciably. For the most active compound **IIf** it was ascertained whether this was fungistatic or fungicidal. Since this compound caused complete inhibition of mycelia growth both in tested as well as reinoculated dishes, hence they were fungicidal.

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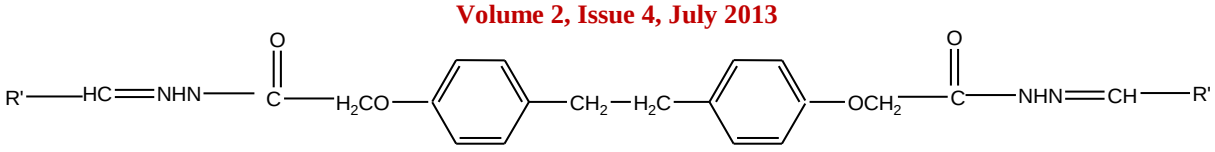
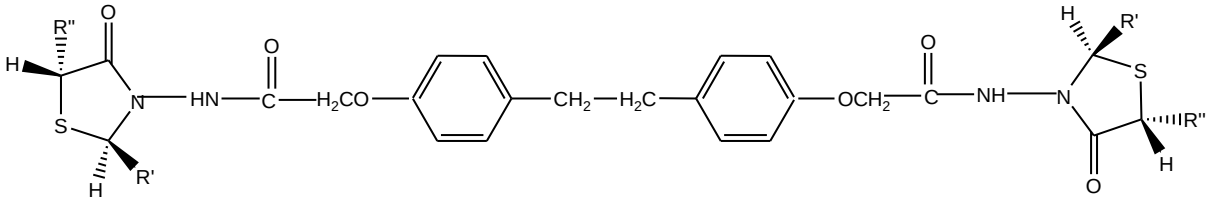


I a-f



IIa-l

Fig1 Synthesized compounds of first series

Cpd.	R’	R”	Cpd.	R’	R”
Ia	3,4-$(CH_3O)_2.C_6H_3$	-	IId	4-$OH.C_6H_4$	H
Ib	4-$CH_3O.C_6H_4$	-	IIe	4-$Cl.C_6H_4$	H
Ic	2-$OH.C_6H_4$	-	IIf	4-$NO_2.C_6H_4$	H
Id	4-$OH.C_6H_4$	-	IIg	3,4-$(CH_3O)_2.C_6H_3$	CH_3
Ie	4-$Cl.C_6H_4$	-	IIh	4-$CH_3O.C_6H_4$	CH_3
If	4-$NO_2.C_6H_4$	-	IIi	2-$OH.C_6H_4$	CH_3
IIa	3,4-$(CH_3O)_2.C_6H_3$	H	IIj	4-$OH.C_6H_4$	CH_3
IIb	4-$CH_3O.C_6H_4$	H	IIk	4-$Cl.C_6H_4$	CH_3
IIc	2-$OH.C_6H_4$	H	IIl	4-$NO_2.C_6H_4$	CH_3

Table 1. Antifungal Screening Results of compounds I-a-f and IIa-l

Average % inhibition after 96hr.against						
compound	**Aspergillus.niger**			**Fusarium.oxysporium**		
	1000ppm	**100ppm**	**10ppm**	**1000ppm**	**100ppm**	**10 ppm**
Ia	**49**	**26**	**13**	**51**	**29**	**15**
Ib	**45**	**20**	**18**	**41**	**32**	**8**
Ic	**53**	**42**	**25**	**56**	**39**	**19**
Id	**61**	**43**	**20**	**53**	**36**	**19**
Ie	**58**	**37**	**22**	**63**	**46**	**23**
If	**63**	**45**	**31**	**69**	**37**	**26**
IIa	**62**	**30**	**15**	**58**	**32**	**18**
IIb	**52**	**34**	**12**	**59**	**31**	**10**
IIc	**70**	**54**	**27**	**68**	**51**	**25**
IId	**60**	**49**	**17**	**64**	**43**	**28**
IIe	**82**	**65**	**43**	**86**	**69**	**42**
IIf	**100**	**82**	**52**	**100**	**78**	**51**
IIg	**69**	**45**	**25**	**65**	**38**	**21**
IIh	**72**	**56**	**40**	**73**	**46**	**24**

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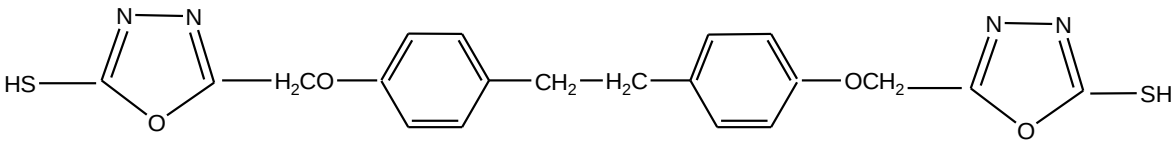
IIi	**75**	**58**	**42**	**76**	**59**	**46**
IIj	**56**	**41**	**22**	**53**	**39**	**19**
IIk	**83**	**67**	**45**	**81**	**65**	**47**
III	**91**	**79**	**51**	**93**	**81**	**50**

Second Series:

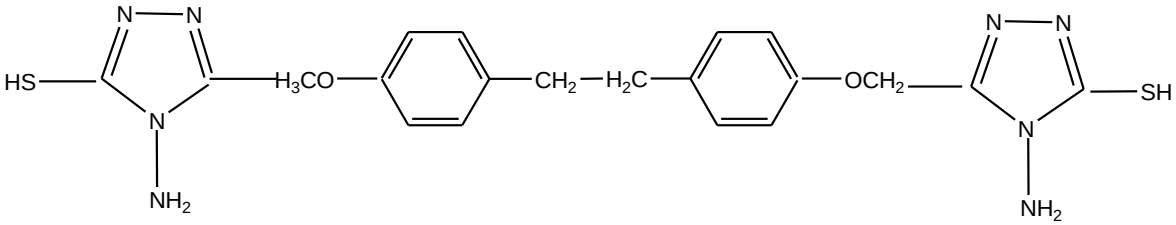
,4,4'-bis[N-(4"-N-benzylidinylamino)-3"mercapto-1",2",4"-triazol-5"-yl-methoxy]bibenzyl

All the synthesized compounds**IIIa-h(fig2)** were screened against A.niger and F.oxysporium for their antifungal activity and the screening data are recorded in **Table2.**It is evident from the screening data that some of the compound have significant fungicidal activity at 1000ppm against both the test fungi but their toxicity decreased considerably at lower concentrations.

For the most active compounds **IIIc** and **IIId** it was ascertained whether they are fungistatic or fungicidal thus ,following the procedure of Garber and Houstaon,compound **IIIc** and **IIId** were added separately to PDA medium at their respective lethal doses(1000ppm and 500ppm).It was found that compound **III**c and **III**d caused complete inhibition of mycelia growth of the test fungi in treated as well as reinoculated dishes and hence were fungicidal.



I



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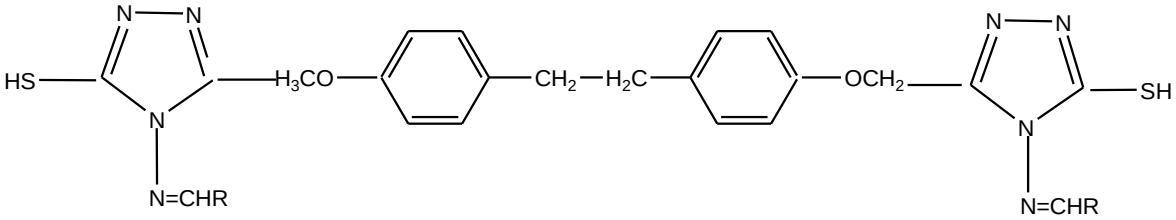

III a-h

Fig 2 Synthesized compound of second series

Compd	R
IIIa	C_6H_5
IIIb	2-OH.C_6H_4
IIIc	4-CH_3O.C_6H_4
IIId	4-Cl.C_6H_4
IIIe	3,4-(CH_3O).C_6H_3
IIIf	4-OH-3-CH_3O.C_6H_3
IIIg	4-NO_2.C_6H_4
IIIh	4-OH.C_6H_4

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Table-2.Antifungal Screening Results of compounds I,II and IIIa-h

Average % inhibition after 96hr.against						
compound	Aspergillus.niger			Fusarium.oxysporium		
	1000ppm	100ppm	10ppm	1000ppm	100ppm	10 ppm
I	47	32	13	51	29	15
II	64	50	24	61	33	20
IIIa	78	52	32	67	50	27
IIIb	73	47	21	69	34	12
IIIc	100	67	53	100	74	51
IIId	93	61	38	87	69	32
IIIe	89	72	29	83	64	22
IIIf	68	44	12	70	49	18
IIIg	72	50	23	74	52	28
IIIh	63	47	20	65	48	30

Third Series:

4,4'-bis[(2"-arylthiazolo[4,3-b]-1,3,4-oxadiazol-5yl)methoxy]bibenzyl ((fig3)

The antifungal activity of compound **Ia-h,IIa-h and IIIa-h(fig3)** were evaluated and the results have summarized in **Table 3**.From the table it is evident that all the tested compound **Ia-h,IIa-h and IIIa-h** displayed significant activity at 1000ppm. Concentration against both test fungi at 1000ppm concentration, but thie activity decreased at lower concentrations. The most active of these **IIe,IIf,IIIb and IIIe** have displayed fungicidal action equivalent to that of commercial fungicide Dithane-M45 at 1000ppm concentration. It was noted that the introduction of methoxy group in the aryl moiety of these compounds tended to augment the antifungal action. Presumably, this was due to lipophilic characters of methoxy group, which favors the permeation of the compound through lipoid barriers in the fungal cell. Further it was found that the introduction of the methoxy group in the aryl moiety decreased the antifungal activity which was probably due to increase in size.

For the highly active compound IIe,IIf, **IIIb and IIIe(fig3)** it was ascertained whether this was fugistatic or fungicidal. Since these compound caused complete inhibition of mycelia growth both in treated as well as in reinoculated dishes hence were fungicidal

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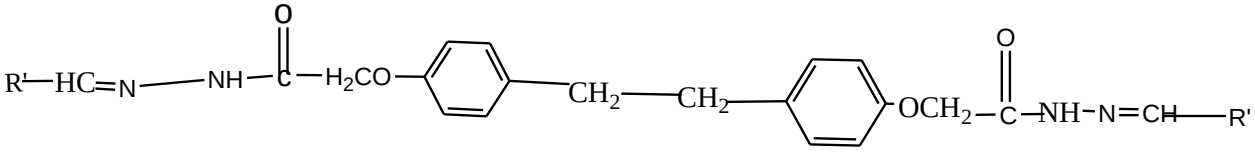
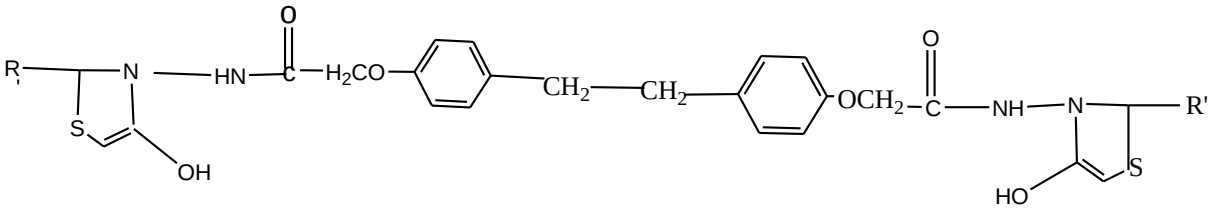
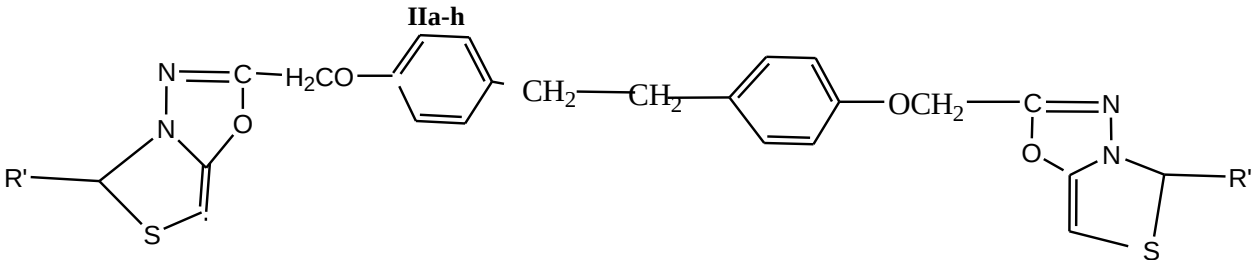
Fig 3 Synthesized compound third series



Ia-h



IIa-h



IIIa-h

Compd.	R’
a	3,4-$(CH_3O)C_6H_3$
b	4-(CH_3O)-C_6H_4
c	2- $OH.C_6H_4$
d	4- $OH.C_6H_4$
e	4- $Cl.C_6H_4$
f	4- $NO_2.C_6H_4$
g	4- OH-$3CH_3O.C_6H_3$
h	C_6H_5

Table 3.Antifungal Screening Results of compounds Ia-h,IIa-h and IIIa-h

Average % inhibition after 96hr.against						
compound	Aspergillus.niger			Fusarium.oxysporium		
	1000ppm	100ppm	10ppm	1000ppm	100ppm	10 ppm
Ia	49	26	13	51	29	15
Ib	45	20	18	41	32	8
Ic	53	42	25	56	39	19
Id	61	43	20	53	36	19
Ie	58	37	22	63	46	23
If	63	45	31	69	37	26

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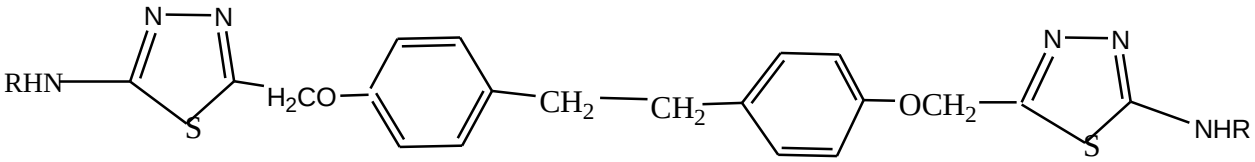
Ig	54	40	22	57	39	21
Ih	63	47	33	65	48	37
IIa	62	30	15	58	32	18
IIb	52	34	12	59	31	10
IIc	70	54	27	68	51	25
IId	60	49	17	64	43	28
IIe	82	65	43	86	69	42
IIf	100	82	52	100	78	51
IIg	72	56	31	71	55	30
IIh	61	38	19	59	34	12
IIIa	73	49	32	71	47	33
IIIb	100	67	51	100	70	53
IIIc	66	43	21	62	40	12
IIId	61	41	24	68	45	18
IIIe	89	53	42	87	55	39
IIIf	71	44	35	75	50	37
IIIg	73	46	39	70	49	26
IIIh	65	38	22	67	34	29

Fourth Series:

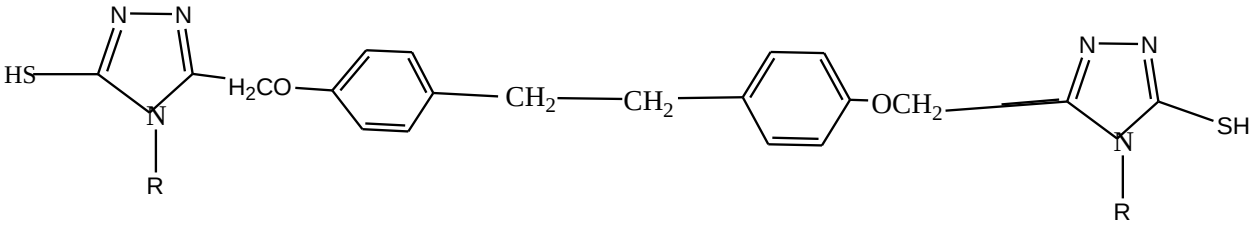
4,4'-bis[2"-(aryl/alkylimino)-4"-oxothiazolin-3"ylacetamidoxy)bibenzyls and4,4'bis(thiadiazolyl/triazolyl/oxadiazolyl methoxy)bibenzyls"(fig4)

All the synthesized **compounds I and IIa-h** were screened against A.niger and F.oxysporium for their antifungal activity and the screening data are recorded in **Table 4**.It is clear from the antifungal screening data that all the screened compound inhibited 63-100% mycelia growth of both the test fungi at 1000ppm concentration but their activity decreased considerably at lower concentration(100,10ppm).The most active of **these IIa IIb and IIc** have displayed fungicidal action equivalent to that of commercial fungicide Dithane M-45 at 1000ppm concentration. From the table it is evident that compound VII is less active than their successors **VIIIa-d.**This has demonstrated that the presence of heterocyclic moieties in these compounds,**IIa-d** enhanced their antifungal activity. It was however noted that compound **IIa** which has thiadiazole nucleus show 100% activity as compared to the other compounds. The order of fungitoxicity of these compounds is **IIa>IIb>IIc>VIId>IIe>IIf(fig4)**. For the most active compounds **IIa,IIb** and **IIc** it was ascertained whether these were fungistatic or fungicidal. Since these compounds caused complete inhibition of mycelia growth in tested as well as in reinoculated dishes, hence were fungicidal.

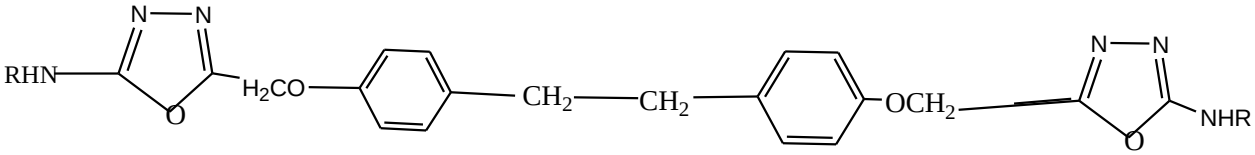
Fig4 Synthesized compound of fourth series



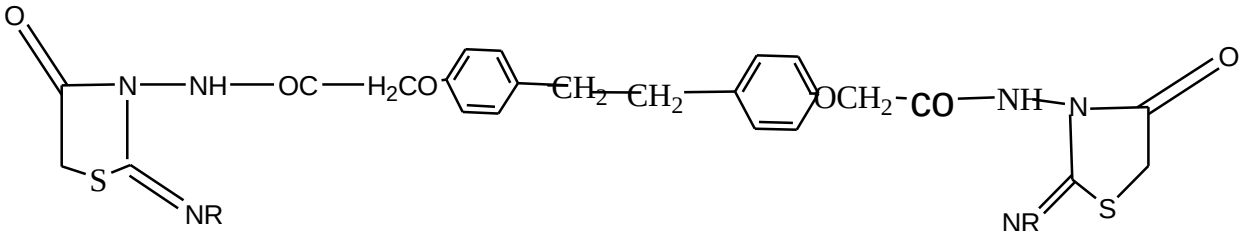
Ia-h



IIa-b



IIc-d



R=C_6H_5,C_2H_5 **IIg-h**

Table-4.Antifungal Screening Results of compounds I&IIa-h

Average % inhibition after 96hr.against						
compound	Aspergillus.niger			Fusarium.oxysporium		
	1000ppm	100ppm	10ppm	1000ppm	100ppm	10 ppm
I	61	50	22	51	29	15
IIa	100	78	51	100	72	47
IIb	91	83	67	90	82	62
IIc	86	58	31	77	49	24
IId	79	53	29	75	49	27
IIe	76	47	23	61	32	18
IIf	71	42	21	68	38	22
IIg	63	35	16	68	40	25
IIh	55	32	19	58	37	23

IV. CONCLUSION

On the basis of the preliminary screening of the compounds for their antifungal activity, the following conclusions may be drawn;

1. The antifungal action may not be numerical sum of several toxophoric functions; perhaps in a congregation of such toxophoric functions, the role of only a few key factors is apparently important.

2. All the screened compounds inhibit the growth of both the test fungi viz: Aspergillus niger and Fusarium oxysporium in the range of 46-100% at 1000ppm. Concentration, hence are antifungal.

3. It was also show that unsubstitued phenyl nuleus is less toxic as compared with that bearing chloro, nitro or methyl, methoxy group.

4.The most active compounds ,**IIc,IIe,IIf,IIk(Table 1),IIIc,IIId,IIIe**(**Table 2),IIIb,IIIe(Table 3) &IIa , IIb and IIc(Table4)** displayed fungicidal activity of the order of Dithane M-45(a commercial fungicide) at 1000ppm and inhibited greater than 50% growth of both the test fungi even at 10ppm concentration. Therefore, these, compounds want further screening at higher dilution and against wide range of fungi. This is under investigation and will be published elsewhere.

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