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Research Article

Synthesis and antifungal activity of 4-substituted aryl methyl-2-(4'-acetylamino-3'-bromophenyl)-1, 3, 4-oxadiazolin-5-thiones and substituted-1, 2, 4-triazolin-5-thiones

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ABSTRACT

Starting material 4-acetylamino-3-bromobenzoyl hydrazine (1) has been synthesized from easily available methyl-4-acetylamino-3-bromobenzoate and hydrazine hydrate in ethanol. Compound (1) react with KOH and CS₂ gives 2-(4'-acetylamino-3'-bromophenyl)-1, 3, 4-oxadiazoline-5-thiones (2). Aminomethylation of (2) with formaldehyde and secondary amines in ethanol furnished Manich base (3a-e). Compound (1) was again treated with aryl'isothiocyanate to get 4-aryl'thiosemicarbazide (4a-d) which underwent cyclization in the presence of NaOH to give corresponding triazolinthione (5a-d). Compound (5a-d) on aminomethylation with formaldehyde and secondary amine in ethanol to give aryl¹-1, 2, 4-triazoline-5-thiones (6a-l). Antfungal activity has been compaired with Dithane M-45, a commercial fungicide, for their fungitoxic action against *Phytophthora infestance* and *Collectotricum falcatum*, and the result correlated with their structural features.

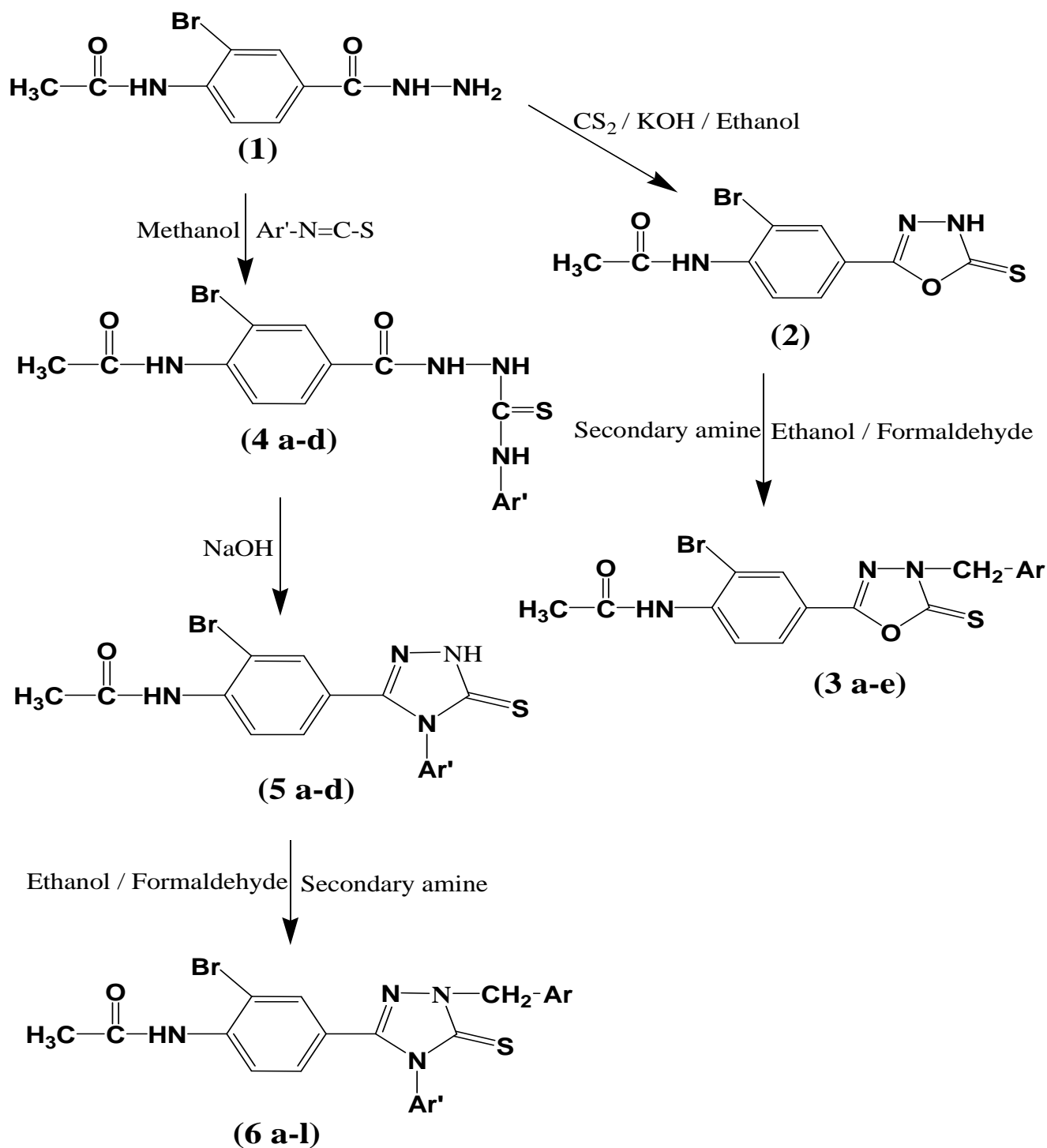
Keywords: Chemo selectivity, Oxadiazole derivatives, Triazole derivative, Fungicidal Activity, Pharmacological properties, Infrared spectra, ¹H NMR spectra.

INTRODUCTION

1,3, 4-Oxadiazolin –5-thiones and 1, 2, 4-triazoline-5-thiones have been reported to exhibit antifungal, antibacterial, antileishmanial and insecticidal activities¹⁻⁶. Keeping such biological activity profile in view compounds, the reaction sequence leading to the formation of title compounds are given in **Scheme-I**.

The intermediate benzoylhydrazine (1) was prepared by the hydrazinolysis of corresponding methyl ester. The hydrazides was cyclized with carbon disulphide and potassium hydroxide⁷ followed by acidification to corresponding 1, 3, 4-oxadiazolin-5- thione (2) which was amino ethylated with formaldehyde and

secondary amines in ethanol to form (3a-e). In another reaction treatment of (1) with aryl¹isothiocyanate to give 4-aryl¹-thiosemicarbazides (4a-d) which in alkaline medium gave triazolinthione (5a-d). Compound (5a-d) on being subjected to Manich reaction in the presence of formaldehyde and secondary amines to give 4-aryl¹-1, 2, 4-triazolin-5-thiones (6a-l). The structures of all new compounds were established on the basis of their spectral data.



Scheme-I

EXPERIMENTAL

All melting point determined in open glass capillaries. All the solvents and reagents used were of analytical grade. All the reactions were monitored by TLC using Benzene: Methanol (9: 2), Methylene dichloride: Ethyl acetate: Methanol (60: 35: 05) and Toluene: Ethyl acetate (7: 3) as a solvent system TLC plates were prepared by spreading method. These were dried in the air and then activated by heating in hot air oven at 110°C for 30 minutes iodine vapors were used for visualization of TLC plates. IR spectra in KBr were recorded on Perkin-Elmer infrared spectrophotometer (λ max in cm^{-1}) and ^1H NMR spectra in DMSO-d_6 on EM-360L (60MHz) NMR Spectrometer using TMS as internal references (Chemical shifts in δ ppm). All the compounds have given satisfactory elemental analysis (C, H, N, and S), IR and ^1H NMR spectra.

4-Acetylamino-3-bromobenzoyl hydrazine (1): A mixture of methyl 4-acetylamino-3-bromobenzoate (0.01 M), hydrazine hydrates (0.01 M, 99%) and Ethanol (20.0 ml) was refluxed for 10.0 hrs. Excess of solvent was distilled off and solid thus obtained was filtered and washed with water and recrystallized from ethanol. Yield 79%, m. p. 176°C.

2-(4¹-Acetylamino-3¹-bromophenyl)-1, 3, 4-oxadiazolin-5-thione (2): A mixture of Compound **1** (0.01 M), potassium hydroxide (0.015 M), carbon disulphide (0.015 M) and ethanol (50.0 ml, 95%) was refluxed for 6.0 hrs. Excess of solvent was distilled off and remaining residue was poured in ice cold water and acidified with acetic acid. The yellow colored compound thus obtained was filtered and dried. Yield 83%, m. p. 238°C.

IR Spectra of Compound 2: IR (KBr): 3445, 3340 (N-H), 1615 (C=O), 1510 (C=N), 1190 (C-O-C), 1045 (C=S), 550 (C-Br) cm^{-1} .

4-Arylinomethyl-2-(4¹-acetylamino-3¹-bromophenyl)-1, 3, 4-oxadiazolin-5-thione (3a-e): To a suspension of compound **2** (0.01 M) in Ethanol (5.0 ml), formaldehyde (1.0 ml, 37%) and secondary amine (0.01 M) was added. The solution become turbid this soon cleared on warming on a water bath for 2.0 mints. Clear solution was left overnight at room temp. The solid product obtained was recrystallized from chloroform-Pet-ether (60-80°) (1:1); all the synthesized five compounds are given in the **Table –1** with their characterization data.

IR and ^1H NMR Spectra of Compound 3a: IR (KBr): 3385, 3375 (N-H), 2848 ($-\text{CH}_2$), 1620 (C=O), 1515(C=N), 1160 (C-O-C), 1060 (C=S), 550 (C-Br) cm^{-1} . ^1H NMR (DMSO-d_6) δ : 1.75 (s, 4H, $-\text{CH}_2\text{-O-CH}_2$), 2.20 (s, 3H, NH-CO-CH_3), 2.75-2.89 (t, 4H, $-\text{CH}_2\text{-N-CH}_2$), 5.20(s, 2H, $>\text{N-CH}_2\text{-N}<$), 5.75 (s, 1H, NH- CO-CH_3), 7.0-8.0 (m, 3H, Ar-H).

IR and ^1H NMR Spectra of Compound 3c: IR (KBr): 3395, 3365 (N-H), 2845 ($-\text{CH}_2$), 1610 (C=O), 1515 (C =N), 1080 (C=S), 1165(C-O-C), 545 (C-Br) cm^{-1} . ^1H NMR (DMSO-d_6) δ : 2.18 (s, 3H, NH-CO-CH_3), 3.96-3.99 (t, 1H, N-H), 5.23 (s, 2H, $>\text{N-CH}_2\text{-N}<$), 5.73 (s, 1H, NH-CO-CH_3), 7.20-7.68 (m, 8H, Ar-H).

IR and ^1H NMR Spectra of Compound 3e: IR (KBr): 3395, 3370 (N-H), 2845 ($-\text{CH}_2$), 1620 (C=O), 1510 (C=N), 1160 (C-O-C), 1046 (C=S), 545 (C-Br) cm^{-1} . ^1H NMR (DMSO-d_6) δ : 2.16 (s, 3H, NH-CO-CH_3), 3.82 (s, 3H, $-\text{OCH}_3$), 3.93-3.98 (t, 1H, NH), 5.26 (s, 2H, $>\text{N-CH}_2\text{-N}<$), 5.75 (s, 1H, NH-CO-CH_3), 7.15-7.82 (m, 7H, Ar-H).

1-(4¹-Acetylamino-3¹-bromophenyl)-4aryl¹thiosemicarbazide (4a-d): To a mixture of compound **1** (0.01 mol) was dissolved in hot methanol (40.0ml). Aryl¹ isothiocyanate (0.01 M) was added to the clear solution and the whole solution was refluxed for 3.0 hrs. The solid compound thus obtained was recrystallized from methanol, resulting in sparkling colorless crystalline compounds; all the prepared four compounds are given in the **Table-2** with their characterization data.

Table-1: Antifungal activity data of 4-arylinomethyl-2(4¹-acetylamino-3¹-bromophenyl)-1, 3, 4-oxadiazolin-5-thiones (3a-e) and 3-(4¹-acetylamino-3¹-bromophenyl)-1-arylinomethyl-4-aryl¹ -1, 2, 4-triazolin-5-thiones (6a-l).

Compd. No.	Average % inhibition against					
	<i>Phytophthora infestance</i> at			<i>Collectotricum falcatum</i> at		
	1000 ppm	100 ppm	10 ppm	1000 ppm	100 ppm	10 ppm
3 a.	99	62	45	98	61	44
b.	88	44	33	87	41	29
c.	98	59	43	98	58	46
d.	87	46	40	86	45	38
e.	85	44	42	84	45	41
6. a.	84	42	34	85	43	33
b.	75	39	28	74	36	29
c.	82	40	32	80	42	30
d.	99	63	45	98	62	44
e.	98	58	49	98	59	48
f.	99	69	53	98	68	52
g.	97	51	42	96	50	41
h.	98	48	40	98	45	38
i.	97	46	38	97	45	39
j.	88	42	32	87	41	31
k.	86	39	30	87	38	30
l.	87	40	34	85	39	32
Dithane M-45	100	84	65	100	83	63

Table-2: Characterization data of 4-arylinomethyl-2-(4¹-acetylamino-3¹-bromophenyl)-1, 3, 4-oxadiazolin-5-thiones (3a-e), 1-(4¹-acetylamino-3¹-bromophenyl)-4-aryl¹ thiosemicarbazides (4a-d), 1-(4¹-acetylamino-3¹-bromophenyl)-4-aryl-1, 2, 4-triazolin-5-thiones (5a-d) and 3-(4¹-acetylamino-3¹-bromophenyl)-1-arylinomethyl-4-aryl¹-1, 2, 4-triazolin-5-thiones (6a-l)

Compd. No.	Ar	Ar ¹	Molecular Formula	m. p. (°C)	Yield (%)	Found (Calcd.) %		
						C	N	S
3 a*.	Morpholino	-	C ₁₅ H ₁₇ O ₃ N ₄ SBr	184	73	43.59 (43.57)	13.57 (13.59)	07.75 (07
b.	Piperidino	-	C ₁₆ H ₁₉ O ₂ N ₄ SBr	172	69	46.72 (46.71)	13.62 (13.63)	07.78 (07
c*.	Anilino	-	C ₁₇ H ₁₅ O ₂ N ₄ SBr	138	74	48.69 (48.66)	13.36 (13.38)	07.63 (07
d.	N-Me-piprazino	-	C ₁₆ H ₂₀ O ₂ N ₅ SBr	173	67	46.61 (46.60)	13.59 (13.58)	07.76 (07
e*.	p-OMe-anilino	-	C ₁₈ H ₁₇ O ₃ N ₄ SBr	150	63	48.11 (48.13)	12.47 (12.46)	07.12 (07
4 a.	-	C ₆ H ₅	C ₁₆ H ₁₅ N ₄ SO ₂ Br	201	70	47.18 (47.16)	13.76 (13.77)	07.86 (07
b.	-	o-ClC ₆ H ₄	C ₁₆ H ₁₄ N ₄ SO ₂ BrCl	198	71	43.49 (43.48)	12.68 (12.69)	07.24 (07
c.	-	o-OCH ₃ C ₆ H ₄	C ₁₇ H ₁₇ N ₄ SO ₃ Br	208	68	46.69 (46.66)	12.81 (12.80)	07.32 (07
d.	-	o-CH ₃ C ₆ H ₄	C ₁₇ H ₁₇ N ₄ SO ₂ Br	203	66	48.46 (48.47)	13.30 (13.32)	07.60 (07
5 a.	-	C ₆ H ₅	C ₁₆ H ₁₃ N ₄ SO Br	229	69	49.37 (49.36)	14.39 (14.41)	08.22 (08
b.	-	o-ClC ₆ H ₄	C ₁₆ H ₁₂ N ₄ SO Br Cl	226	70	45.34 (45.36)	13.22 (13.23)	07.55 (07
c.	-	o-OCH ₃ C ₆ H ₄	C ₁₇ H ₁₅ N ₄ SO ₂ Br	236	67	48.69 (48.66)	13.36 (13.38)	07.63 (07
d.	-	o-CH ₃ C ₆ H ₄	C ₁₇ H ₁₅ N ₄ SO Br	232	68	50.63 (50.62)	13.89 (13.87)	07.94 (07
6.a *	Morpholino	C ₆ H ₅	C ₂₁ H ₂₂ O ₂ N ₅ SBr	176	74	53.40 (53.43)	14.83 (14.85)	06.78 (06.
b.	Piperidino	C ₆ H ₅	C ₂₂ H ₂₄ ON ₅ SBr	163	64	54.33 (54.32)	14.40 (14.42)	06.58 (06.
c.	Anilino	C ₆ H ₅	C ₂₃ H ₂₀ ON ₅ SBr	168	63	62.74 (62.75)	15.91 (15.90)	07.27 (07.
d.	Morpholino	o-ClC ₆ H ₄	C ₂₁ H ₂₁ O ₂ N ₅ SBrCl	132	62	48.23 (48.22)	13.39 (13.40)	06.12 (06.
e.*	Peperidino	o-ClC ₆ H ₄	C ₂₂ H ₂₃ ON ₅ SBrCl	167	70	50.73 (50.74)	13.45 (13.43)	06.14 (06.
f.	Anilino	o-ClC ₆ H ₄	C ₂₃ H ₁₉ ON ₅ SBrCl	160	68	52.23 (52.21)	13.24 (13.26)	06.05 (06.
g.	Morpholino	o-OCH ₃ C ₆ H ₄	C ₂₂ H ₂₄ O ₃ N ₅ SBr	162	63	50.97 (50.96)	13.51 (13.50)	06.17 (06.
h.	Anilino	o-OCH ₃ C ₆ H ₄	C ₂₃ H ₂₆ O ₂ N ₅ SBr	160	64	53.49 (53.51)	13.56 (13.54)	06.20 (06.
i.*	Peperidino	o-OCH ₃ C ₆ H ₄	C ₂₄ H ₂₂ O ₂ N ₅ SBr	168	66	54.97 (54.95)	13.36 (13.37)	06.10 (06.
j.	Morpholino	o-CH ₃ C ₆ H ₄	C ₂₂ H ₂₄ O ₂ N ₅ SBr	169	65	52.60 (52.61)	13.94 (13.95)	06.37 (06.
k.	Peperidino	o-CH ₃ C ₆ H ₄	C ₂₃ H ₂₂ ON ₅ SBr	162	62	55.65 (55.64)	14.11 (14.13)	06.45 (06.
l.*	Anilino	o-CH ₃ C ₆ H ₄	C ₂₄ H ₂₂ ON ₅ SBr	166	66	56.70 (56.73)	13.78 (13.76)	06.30 (06.

3-(4¹-Acetylamino-3¹-bromophenyl)-4-aryl¹-1, 2, 4-triazolin-5-thiones (5a-d): To a mixture of compound **4** (0.005 M) was dissolved in sodium hydroxide solution (20.0 ml, 5%), the resulting brown solution was refluxed for 3.0 hrs, cooled and neutralized with dilute HCl. Brilliant off white crystalline product so obtained was washed several times with cold water and recrystallized from ethanol; all the synthesized four compounds are given in the **Table-2** with their characterization data.

3-(4¹-Acetylamino-3¹-bromophenyl)-1-arylinomethyl-4-aryl¹-1,2,4-triazolin-5-thione(6a-l):

Formaldehyde (1.0 ml, 37%) and Secondary amine (0.01 M) was added to a suspension of **5** (0.01 M) in ethanol (5.0 ml). Turbid solution so obtained soon become clear on warming on a water bath for 2.0 mints and left overnight at room temperature colorless solid product, thus obtained was recrystallized from Chloroform-Pet ether (60-80°) (1:1), all the synthesized twelve compounds are given in the **Table-2** with their characterization data.

IR and ¹H NMR Spectra of Compound 6a: IR (KBr): 3374 (N-H), 2855 (-CH₂-), 1610 (C=O), 1500 (C=N), 1175 (C-O-C), 1070 (C=S), 545 (C-Br) cm⁻¹. ¹H NMR (DMSO-d₆) δ: 2.19 (s, 3H, NH-CO-CH₃), 2.75-2.90 (t, 4H, -CH₂-N-CH₂), 3.66-3.80 (t, 4H, -CH₂-O-CH₂), 5.20 (s, 2H, >N-CH₂-N<), 5.75 (s, 1H, NH-CO-CH₃), 7.2-7.8 (m, 8H, Ar-H).

IR and ^1H NMR Spectra of Compound 6e: IR (KBr): 3385 (N-H), 2855 ($-\text{CH}_2-$), 1615 (C=O), 1510 (C=N), 1070 (C=S) cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 2.15 (s, 3H, NH-CO-CH₃), 2.78-2.95 (t, 4H, $-\text{CH}_2-\text{N}-\text{CH}_2-$), 3.69-3.85 (t, 6H, $-\text{CH}_2-\text{CH}_2-\text{CH}_2$), 5.21 (s, 2H, $>\text{N}-\text{CH}_2-\text{N}<$), 5.75 (s, 1H, NH-CO-CH₃), 7.2- 8.0 (m, 7H, Ar H).

IR and ^1H NMR Spectra of Compound 6i: IR (KBr): 3395 (N-H), 2852 ($-\text{CH}_2-$), 1630 (C=O), 1525 (C=N), 1095 (C=S) cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 2.17 (s, 3H, NH-CO-CH₃), 3.80 (s, 3H, $-\text{OCH}_3$), 3.92-3.99 (t, 1H, NH), 5.23 (s, 2H, $>\text{N}-\text{CH}_2-\text{N}<$), 5.80 (s, 1H, NH-CO-CH₃), 7.1-7.89 (m, 12H, Ar-H).

IR and ^1H NMR Spectra of Compound 6l: IR (KBr): 3385 (N-H), 2850 ($-\text{CH}_2-$), 1615 (C=O), 1510 (C=N), 1060 (C=S) cm^{-1} . ^1H NMR ($\text{DMSO}-d_6$) δ : 2.18 (s, 3H, NH-CO-CH₃), 3.40 (s, 3H, $-\text{CH}_3$), 3.90-3.95 (t, 1H, NH), 5.26 (s, 2H, $>\text{N}-\text{CH}_2-\text{N}<$), 5.83 (s, 1H, NH-CO-CH₃), 7.0-8.1 (m, 12H, Ar-H).

ANTIFUNGAL ACTIVITY

The antifungal activity of the compounds (**3a-e**) and (**6a-l**) were evaluated against *Phytophthora infestans* and *Collectotricum falcatum* by usual agar plate technique⁸ at 1000, 100 and 10 ppm concentrations⁹⁻¹³.

Dithane M-45 a standard commercial fungicide was also tested under similar conditions for comparison. The antifungal activity results of the compounds (**3a-e**) and (**6a-l**) are summarized in **Table -1**. It is appeared from the screening results that most of the compounds (**3a-e**) and (**6a-l**). Significantly inhibited the mycelia growth of both test fungi at 1000 ppm but their activity decreased considerably at lower concentration (100 and 10 ppm). The compounds **3a,3c,6d,6e,6f** had similar activity to mancozed at 1000 ppm and showed **53-44%** growth inhibition of both the test fungi at 10 ppm concentration. It was significant alteration in the antifungal activity with the change in the relative position of the substituent on oxadiazoline and triazoline rings, for example compounds **6d, 6e, 6f** bearing 2-chloro group were more active than **6g, 6h, 6i** 2-methoxy groups. Likewise, introduction of the chloro group was for more effective than that at methoxy group.

RESULT AND DISCUSSIONS

The new oxadiazolin-5-thiones (**3 a-e**) and triazolin-5-thiones (**6 a-l**) was prepared from benzoylhydrazine (**1**). Compound (**1**) react with KOH and CS₂ gives 2-(4¹-acetylamino-3¹-bromophenyl)-1, 3, 4-oxadiazoline-5-thiones (**2**). Aminomethylation of (**2**) with formaldehyde and secondary amines in Ethanol furnished Manich base (**3a-e**). Compound (**1**) was again treated with aryl¹isothiocynate to get 4-aryl¹thiosemicarbazide (**4a-d**) which underwent cyclization in the presence of NaOH to give corresponding triazolinthione (**5a-d**). Compound (**5a-d**) on aminomethylation with formaldehyde and secondary amine in ethanol to give aryl¹-1, 2, 4-triazoline-5-thiones (**6a-l**). The structures of the compounds were confirmed by their melting points, elemental analysis, IR spectra and position in ^1H NMR spectra.

CONCLUSION

It is appeared from screening results the most of the compounds (**3a-e**) and (**6a-l**) significantly inhibited the mycelia growth of both test fungi at 1000 ppm but their activity decreased considerably at lower concentration (100 and 10 ppm). The compounds **3a, 3c, 6d, 6e, 6f** had similar activity at 1000 ppm and showed 53-44% growth inhibition of both test fungi at 10 ppm concentration. Significant alteration of the fungicidal activity was observed with the change in the relative position of the substituent on Oxadiazole-5-thiones and Triazole-5-thiones with Acetylamino-bromophenyl ring e. g. compounds **6d, 6e, 6f** bearing 2-chloro group were more active than **6g, 6h, 6i** 2-methoxy groups. Likewise, introduction of the chloro group was for more effective than that at methoxy group.

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