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In-Vitro Evaluation of Novel Synthetic Compounds against Alternaria *brassicicola*

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ABSTRACT

A total of seven Pyrazolones and Pyridines and three Schiff bases (C1-C10) were evaluated *in-vitro* against *Alternaria brassicicola* causative organism of black spot disease among crucifers. The pyridine based compounds were found superior to Schiff base compounds against .*Alternaria brassicicola.* Two novel synthetic compounds viz., 2-Benzyl Pyridine (**C10**), and 2-N-[Salysaldehyde] amino pyridine (**C9**) recorded significant anti-microbial activity against *Aternaria brassicicola.*

Key words: *Atermaria brassicicola*, black spot, Pyrazolones, Pyridines and Schiff bases

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INTRODUCTION

Plant diseases are known since ancient times and fossil evidence indicates that plants were affected by diseases 250 million years ago. There are many examples of plant diseases that have made impact on society and have been changed human history [1]. Loss of crops from plant diseases may results in hunger and starvation, especially in less developed countries where access to disease control method is limited and annual losses of 30 to 50% are common for major crops like mustard in countries like India.

Mustard *(Brassica campestris)* comes under the family Cruciferae (Brassicaceae). Mustard as well as other crucifers is affected by different species of fungal phytopathogens. Different species of pathogens of *Alternaria family are responsible for black leaf spot disease infection in four species of crucifers.*viz; 1. *Brassica campestris* (Mustard) infected by *Alternaria brassicicola* 2. *Brassica oleraceae* (Patta gobhi) infected by *Alternaria brassicicola* 3. *Brassica nigra* (Black mustard / kali rai) infected by *Alternaria brassicicola* and4. *Raphanus sativus* (Raddish) infected by *Alternaria brassicicola*[3, 4].

Several synthetic as well as natural compounds were screened against these microbial diseases by several workers. Synthetic compounds available in market to control fungal phytopathogenic diseases include- Biltox, Mancozeb (Dithane M-45), Maneb and Thiram for *Alternaria brassicicoloa*, (Black or brown spot disease in Mustard and crucifers) [2, 3].

In the present study, ten novel synthetic organic compounds in the series of Pyrazolones, Pyridines and Schiff bases were selected for *in-vitro* antifungal study against the mustard phytopathogen- *Alternaria brassicicoloa*. The selection of ten novel synthetic organic compounds was based on the fact that none of these compounds were not previously screened against this selected phytopathogen [4]**.** The whole *in-vitro* antifungal study was focused to find out the solution for pest management of *Alternaria brassicicoloa* on crucifers (Mustard) crop of local area.

MATERIAL AND METHODS

FUNGAL CULTURE

Plant pathogen *Alternaria brassicicoloa* (ITCC-2939) were procured from division of plant pathology, I.A.R.I. New Delhi. The test organism were cultured on Potato dextrose agar (PDA) and Potato dextrose broth (PDB) and incubated at 22-24 ºC for 5-7 days for revival and sub culturing of test organism. For inoculation 100µl. of actively growing cells of (OD 10^6–10^8/ ml.) *Alternaria brassicicoloa* inoculated Potato dextrose broth (PDB) was used to inoculate sterile Potato dextrose agar plates through spread plate swab method.

SYNTHETIC COMPOUNDS

The ten novel synthetic compounds of three groups viz: Pyrazolones, Pyridines and Schiff bases (C1-C10) as listed out in Table 1 synthesized in the laboratory and their structures were confirmed by standard analytical techniques. The Structures and some physical properties of Pyrazolones, Pyridines and Schiff bases compounds are listed out in Table.1

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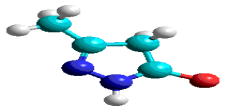
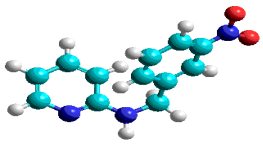
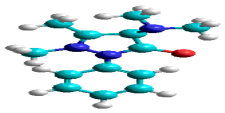
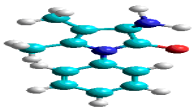
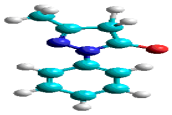
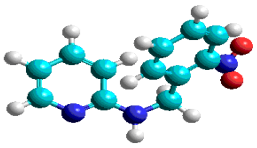
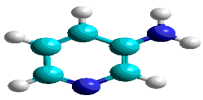
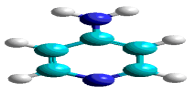
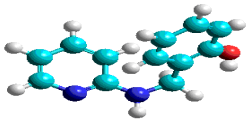
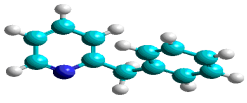
Table. 1

Codes	Name of compounds	Molecular Weights	M. pt. / b. pt. 0ºc	Structures (Ball and Stick models)
1	2	3	4	5
C1 (MeP)	3-Methyl-5-Pyrazolone	70	224 - 226	
C2 (3-N-BAPy)	2-N-[3 Nitrobanzalidene] amino Pyridine	227	202 - 205	
C3 (AMPy)	Amino Pyrine	231	107 - 108	
C4 (4-AAPy)	4-Amino anti-Pyrine	203	107 - 109	
C5 (MPP)	3-Methyl-1-Phenyl-5-pyrazolone	174	124 - 126	
C6 (2-N-BAPy)	2-N-[2-Ntro benzalidene] amino pyridine	227	172 - 177	
C7 (3-AMPy)	3-Amino Pyridine	94	60 - 63	
C8 (4-AMPy)	4-Amino Pyridine	94	157 - 160	
C9 (2-N-SAPy)	2-N-[Salysaldehyde] amino pyridine	198	190 - 192	
C10 (2-BzPy)	2-Benzyl Pyridine	169	140 - 143	

Preparation of inoculums

After 5-7 days of incubation at 22-24 ºc, the test organism *Alternaria brassicicola* was revived. A loopful of culture was taken and dispensed in 50 ml. of Potato dextrose broth (PDB) in a conical flask and kept at 22-24 ºc for further 5-7 days for the preparation of inoculums [8, 9, 10, 11]

Preparation of media and Petri plates

Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB) was used. Media were cooled and then plates were prepared by dispensing 15-20 ml. media per plate. Plates were kept in the same position for ½ hour for solidification to take place and kept inverted in the incubator at 22-24 ºc over night for sterility checking.

Preparation of antimicrobial discs

Whatman 1 filter paper discs of 6 mm. diameter were punched out from barge sheet and were autoclaved at 121ºC, at 15 lbs for 15 minute[12].

Preparation of serial dilutions of compounds

Each compound was 10 times serially diluted (50 mg.→05 mg.) in DMF (Table-2) and each dilution of each compound was tested against fungal phytopathogen.

Table: 2 Concentrations of the test compounds used in the study

Dilutions	Test tube No.	Concentration of Compounds
D0	1	Negative control
D1	2	5 mg.
D2	3	10 mg.
D3	4	15 mg.
D4	5	20 mg.
D5	6	25 mg.
D6	7	30 mg.
D7	8	35 mg.
D8	9	40 mg.
D9	10	45 mg.
D10	11	50 mg.

Inoculation and incubation

100 µl of actively growing cells of (OD 10^6-10^8/ml.) *Alternaria brassicicola* (ITCC-2939) Potato Dextrose Broth (PDB) was used to inoculate sterile Potato Dextrose Agar (PDA) plates through spread plate swab method**.** The whatmann paper discs were dispensed on glass plates

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and each was load with 5µl. volume of pre designated dilution **(C1→C10).** The discs were left air dried in the laminar air flow and were then carefully transferred to inoculated plates at pre designated positions. The plates were then incubated at 22-24 ºC for 5-7 days [13].

RESULTS AND DISCUSSION

Antifungal activity of Pyrazolones and Pyridines

A total of seven Pyrazolones and Pyridines viz; 3-Methyl-5-Pyrazolone (C1), Amino Pyrine (C3), 3-methyl-1-Phenyl-5-pyrazolone (C5), 3-Amino Pyridine (C7), 4-Amino Pyridine (C8), 4-Amino anti-pyrine (C4) and 2-Benzyl Pyridine (C10) were tested for anti-fungal activity against *Alternaria brassicicola.* Of the seven compounds tested 2-Benzyl Pyridine (C10) was found highly effective (Table-3) at the all concentrations tested (5.0 mg to 50 mg.),while 3-Methyl-5-Pyrazolone (C1), Amino Pyrine (C3), 3-methyl-1-Phenyl-5-pyrazolone (C5), 3-Amino Pyridine (C7), 4-Amino Pyridine (C8) and 4-Amino anti-pyrine (C4) did not show antifungal activity against *Alternaria brassicicola.*

Table: 3 Antifungal activities of Pyrazolones and Pyridines against *Alternaria brassicicola*

Concen. → / ↓Compd.	10 ml DMF	5 mg	10 mg	15 mg	20 mg	25 mg	30 mg	35 mg	40 mg	45 mg	50 mg
C1	0.0	-	-	-	-	-	-	-	-	-	-
C3	0.0	-	-	-	-	-	-	-	-	-	-
C5	0.0	-	-	-	-	-	-	-	-	-	-
C7	0.0	-	-	-	-	-	-	-	-	-	-
C8	0.0	-	-	-	-	-	-	-	-	-	-
C4	0.0	-	-	-	-	-	-	-	-	-	-
C10	0.0	**8. 0**	**9. 0**	**9. 0**	**9. 0**	**9. 0**	**9. 0**	**9. 0**	**10**	**10**	**10**

Note: The figures given are zone of inhibition (ZOI) in mm.

Antifungal activity of Schiff bases

A total of three Schiff bases viz; 2-N-[3-Nitrobanzalidene] amino Pyridine (C2), 2-N-[2-nitrobenzalidene] amino pyridine (C6) and 2-N-[Salysaldehyde] amino pyridine (C9) were tested for anti-fungal activity against *Alternaria brassicicola*. Of the three Schiff bases tested 2-N-[Salysaldehyde] amino pyridine (C9) was found highly effective at higher concentration i.e. 40-50 mg. [Table-4] and2-N-[2-nitrobenzalidene] amino pyridine (C6) least effective, while 2-N-[3-Nitrobanzalidene] amino Pyridine (C2) did not show antifungal activity against *Alternaria brassicicola*.

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Table: 4 Antifungal activities for Schiff bases against *Alternaria brassicicola*

Concen. → / ↓Compd.	10ml DMF	5mg	10mg	15mg	20mg	25mg	30mg	35mg	40mg	45mg	50mg
C2	0.0	-	-	-	-	-	-	-	-	-	-
C6	0.0	-	-	-	0. 5	0. 5	0. 5	0. 5	0. 5	0. 5	0. 5
C9	0.0	-	-	-	-	-	-	-	2. 0	2. 0	2. 0

Note: The figures given are zone of inhibition (ZOI) in mm.

The present *in-vitro* antifungal investigation clearly demonstrates the significant antifungal activity of 2-Benzyl Pyridine (C10),2-N-[Salysaldehyde] amino pyridine (C9) against *Alternaria brassicicola*. The results of *in-vitro* antifungal study indicate potential use of these compounds in management of soil born fungal diseases caused by *Alternaria brassicicola*, since phytopathogenic fungi cause diseases in many important crop plants of Crucifers including Mustard.

There is no study available in literature on these synthetic compounds with anti-fungal activity against *Alternaria brassicicola*. This is the first study evaluating the anti-fungal activity of Pyrazolones, Pyridines and Schiff bases Compounds. The study however needs to be substantiated further in field conditions to fully elucidate its potential against plant pathogen.

Analysis and Characterization of Compouunds

Analytical studies of Compounds

Establishment of these compounds was carried out on some physicochemical studies viz: melting point (M. pt.) determination, CHN analysis spectral studies including IR, Mass and NMR spectral studies [14],The preliminary investigation of the compounds viz: m. pt. / b. pt. determination, elemental analysis viz: CHN analysis of Compounds noted on usual methods in Chemistry Research Laboratory, Department of Chemistry, Govt. K.R.G. P.G. Autonomous college Gwalior M.P. CHN analysis of these compounds were carried out on Elemental analyzer Elemental Vario EL III, IR - Perkin Elmer Infrared Spectrophotometer in the range 4000 to 50m^{-1}, Mass - Mass Spectrometers Jeol SX-102(FAB) and NMR spectral studies recorded on NMR Spectrometer Bruker DRX-300 at SAIF CDRI Lucknow [15, 16, 17].

Mass spectral studies of the Compounds

Mass spectral studies of the compounds help in establishing the compounds by means of into fragmentation studies [24]**.** Apart from it studies of parent ion peak in mass spectra of any compound help in the establishment of into molecular weight[18]**.**

Mass spectral studies of the compounds chosen for this study show that the parent ion peaks in the spectra of these compounds appear at the m/e values where these are expected to come. The parent ions peaks for C1, C2, C3, C4, C5, C6, C7, C8, C9, C10 appears at ca 98, 231, 203, 174, 95 , 95, 170, 228, 228, 199. This confirms the molecular weights of the compounds C1, C2, C3, C4, C5, C6, C7, C8, C9, & C10 as compared to their formula weights[21, 22].

Infrared spectral studies

Infrared absorption studies of pyrazolones and pyridine have been assigned by a comparison of these spectra with those of Pyrazole, five membered ring systems [23] the mono-substituted benzene ring system [24] and with those already reported. [25, 26,28, 30].

The C=O stretching frequencies occur at 1614.3, 1661, 1665 & 1598 respectively in case of MeP, AmPy, 4-AAPy & MPP [25, 32]. The strong band at ca has been assigned to the ring stretching of 5 membered ring in Pyrazolone compounds five membered ring hetero atomic compounds are found to have two strong bands near 1590-1560 and 1450-1430 cm^{-1} Which are considered to be characteristic of five membered ring[33, 34]. The bands assigned to the benzene several other absorptions associated with C-H out of plane deformation modes appear in the region 980-840 Cm^{-1} in Pyrazolones[31-33].

Infrared absorption of all Schiff bases has been assigned by comparison of their spectra with those of the five membered Pyrazole ring system [23] the mono substituted benzene ring system [24] and substituted anti-pyrines[26, 31-32]. The notable peaks in case of Schiff base appear at ca 1604-1598 Cm-1 which are attributed to (C=N-) the azomethenic group frequencies. All other diagnostic peaks such as ring stretching, N- phenyl stretching ע (C-N), ring breathing of benzene, C-N-C bending and other deformation peaks matched well with the previous observations [35-37].

Computational Studies of the Compounds

The most common and popular Computational (Semi-empirical) methods used today in the field of Chemi-informatics are MNDO, ZINDO, MNDO/3 AM1 and PM3 etc. In this paper we wish to report AM1, PM3, MNDO and ZINDO calculations for compounds under study. The AM1, PM3, MNDO and ZINDO, Hyperchem 8.0 software package were used to calculate the structure activity relationship (SAR) related parameters such as Heat of formation (HF), Zero point energy (ZPE) Dipole moment (DM), Hydration energy (HE), Refractivity (RF), Polarizability (PZ), Surface area approx (SAA), Surface area Grid, (SAG) and Volume (VOL). All these calculations were carried out on Pentium core-2 Duo machine configuration with windows-Microsoft windows XP[38-40]. The lab reported antimicrobial data have been converted into Log values for SAR analysis (p MIC). All the computed parameters / descriptors were taken as dependent variable step wise regression analysis method [41- 48] was used to develop the SAR equationstatistical parameters such as Correlation coefficient (CC), Standard error (SD), and Fischer test (F-test) etc. were considered to select best SAR Model. When set of parameters / descriptors were subjected to stepwise linear

regression analysisin order develop the SAR equations with different values of CC, SD and F-test. In present study following significant SAR equation were obtained.

p (MIC) = (-0.15691) DM + 0.502872
N = 7, SD = 1.553676, CC = 0.85493, F-test = 0.000329

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