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ARTICLE

Schiff Bases as Potential Fungicides and Nitrification Inhibitors

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A number of substituted Schiff bases were synthesized and characterized by ^{1}H NMR and mass spectrometry. These compounds were screened for antifungal activity *in vitro* against pathogenic fungi, namely, *Sclerotium rolfsii* and *Rhizoctonia bataticola*, and for their effect on nitrification inhibition under laboratory conditions. Maximum antifungal activity was exhibited by (2,4-dichlorobenzylidene)-(2,4,5-trichlorophenyl)-amine and (3-nitrobenzylidene)-(2,4,5-trichlorophenyl)-amine against both fungi (ED_{50} with range from 3 to 24 μg/mL). Maximum nitrification inhibition (NI) was exhibited by (2,4-dichlorobenzylidene)-(2-fluorophenyl)-amine, (4-fluorophenyl)-(3-nitrobenzylidene)-amine, (2,6-dichlorobenzylidene)-(4-fluorophenyl)-amine, and (2,6-dichlorobenzylidene)-(3 fluorophenyl)-amine (NI in the range 91−96%).

KEYWORDS: Schiff bases; antifungal activity; nitrification inhibition

INTRODUCTION

Several pathogenic fungi reduced many food and cash crops up to 20%. These fungi infect seeds, seedlings and mature plants in the field causing collar rot, wilt, damping off, dry root rot (*1*). Among these *Sclerotium rolfsii* and *Rhizoctonia bataticola* are the most devastating soil borne fungi. A large number of chemical protectants used to control these organisms are detrimental to the environment and human health; therefore there is an urgent need to replace these chemical protectants by safe and biodegradable products.

Another major problem of global concern is the low yields of crops due to low efficiency of fertilizer inputs, which results in 16 billion US dollar annual loss of nitrogen (N) fertilizers worldwide (*2*). The factors contributing to N-losses are mainly ammonia volatilization, nitrification and denitrification, and nitrate-leaching. These processes contribute to various health and environmental hazards such as methemoglobinemia in infants, global warming and depletion of the ozone layer in the atmosphere (*3*, *4*). Rapid nitrification is one of the key factors of N-losses. Regulation of urea hydrolysis and nitrification in agricultural system has been one of the major strategies in overcoming these losses. The use of nitrification inhibitors minimizes these effects. Nitrapyrin, dicyandiamide, etridiazole etc. are common commercial nitrification inhibitors. The high cost of development and subsequent registration of effective inhibitors are serious issues in their extensive use (*5*), underlining a need to develop simple, efficient, economical, and safe nitrification inhibitors.

In recent years Schiff bases have been used more frequently for the betterment of both human welfare and agricultural systems due to their wide spectrum of biological activity such as cytotoxicity, anticancer, antifungal, and herbicidal activity (*6*−*9*) and also nitrification inhibitory activity (*10*). The imines and polyamines based on the structure of spermine and spermidine are known to have antiparisitic, antimicrobial and antitubercular activity (*11*). In order to search new biodegradable fungicides and nitrification inhibitors with a broad spectrum of activity, we report herein the synthesis, antifungal and nitrification inhibitory activity of a series of Schiff bases.

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MATERIALS AND METHODS

Chemicals and Instruments. All the chemicals used were purchased from Sigma-Aldrich and used without further purification. Reactions were monitored by thin layer chromatography (TLC) on precoated Merck silica gel $60F_{254}$; the spots were visualized either by UV or by iodine vapor and further purified by column chromatography. Melting points were determined on a melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer model 2000 FT-IR spectrophotometer as KBr pellets, and values are expressed as ν_{max} cm^{-1}. Mass spectral data were recorded on a Joel (Japan) JMS-DX303 and micro mass LCT, mass spectrometer/data system. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Spectrospin spectrometer (300 and 75.5 MHz), using tetramethylsilane as an internal standard. The chemical shift values are recorded on the δ scale, and the coupling constants (J) are in hertz. Elemental analysis for all compounds was performed on a Carlo Erba model EA-1108 elemental analyzer, and data of C, H and N were within ±0.4% of calculated values.

Synthesis. *Substituted Monoimines and Diimines* (***1***−***23***). To a stirred solution of substituted benzaldehyde (5 mmol) in methanol, substituted amines (5.5 mmol) were added and stirred for 3 h (**Figure 1**). The precipitate thus obtained was filtered, washed with minimum amount of cold methanol and recrystallized from ethanol. In the case of diimines, 10 mmol of substituted benzaldehydes was used (**Figure 2**).

(*2,4-Dichlorobenzylidene*)(*4-fluorophenyl*)*amine* (***1***) *(12)*. Yield: 65%; white solid. Melting point: 106 °C. IR: 2923, 1617, 1463, 1099, 740. ^{1}H NMR: 7.05−7.13 (m, 1H, H-5-phenyl), 7.14 (m, 1H, H-3-phenyl), 7.18−7.20 (m, 1H, H-6-phenyl), 7.21−7.29 (m, 1H, H-2-phenyl), 7.33 (d, J = 1.6 Hz, 1H, H-3-benzylidene), 7.35 (dd, J = 0.80, 5.20 Hz, 1H,

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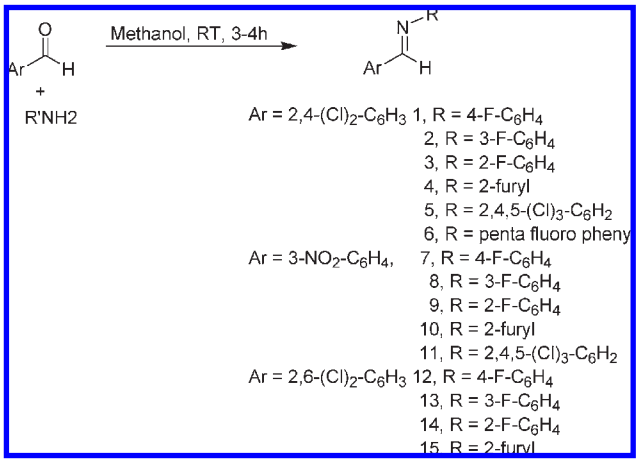

Figure 1. Synthesis of substituted monoimines.

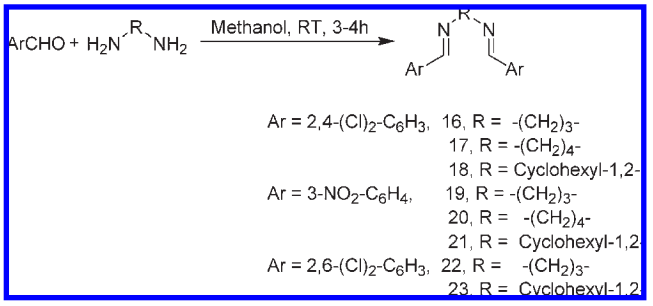

Figure 2. Synthesis of substituted diimines.

H-5-benzylidene), 8.22 (d, J = 8.8 Hz, 1H, H-6-benzylidene), 8.89 (s, 1H, N = CH). HRMS calculated for $C_{13}H_8Cl_2FN$: 268.0018, found 268.1136 (M^+).

(*2,4-Dichlorobenzylidene*)(*3-fluorophenyl*)*amine* (**2**). Yield: 74%; white solid. Melting point: 85 °C. IR: 3026, 1615, 1462, 1099, 722. ^{1}H NMR: 6.62−7.02 (m, 3H, H-2, H-4, H-5-phenyl), 7.32−7.40 (m, 2H, H-6-phenyl, H-5-benzylidene), 7.46 (d, J=2 Hz, 1H, H-3-benzylidene), 8.20 (d, J=9 Hz, 1H, H-6-benzylidene), 8.85 (s, 1H, N=CH). HRMS calculated for $C_{13}H_8Cl_2FN$: 268.0018, found 268.1121 (M^+).

(*2,4-Dichlorobenzylidene*)(*2-fluorophenyl*)*amine* (**3**). Yield: 76%; White solid. Melting point: 102 °C. IR: 3029, 1620, 1463, 1099, 741. ^{1}H NMR: 7.07−7.13 (m, 2H, H-3, H-5-phenyl), 7.22−7.25 (m, 2H, H-4, H-6-phenyl), 7.33−7.35 (d, J = 1.4 Hz, 1H, H-3-benzylidene), 7.42−7.46 (m, 1H, H-5-benzylidene), 8.19 (d, J=8 Hz, 1H, H-6- benzylidene), 8.83 (s, 1H, N=CH). HRMS calculated for $C_{13}H_8Cl_2FN$: 268.0018, found 268.0231 (M^+).

(*2,4-Dichlorobenzylidene*)*furan-2-ylamine* (**4**). Yield: 83%; yellow solid. Melting point: 50 °C . IR: 3076, 1466, 1096, 736. ^{1}H NMR: 6.27−6.28 (m, 1H, H-5-furyl), 6.34−6.36 (m, 1H, H-4-furyl), 7.24−7.28 (m, 2H, H-3-furyl, H-5-benzylidene), 7.39 (d, J = 1.8 Hz, 1H, H-3-benzylidene), 7.90 (d, J=8.20 Hz, 1H, H-6-benzylidene), 8.71 (s, 1H, N=CH). HRMS calculated for $C_{11}H_7Cl_2NO$: 238.9705, found 240.0865 (M^+ + H).

(*2,4-Dichlorobenzylidene*)(*2,4,5-trichlorophenyl*)*amine* (**5**). Yield: 65%; yellow color solid. Melting point: 118 °C. IR: 2955, 1621, 1583, 1470, 1369, 1079, 732. ^{1}H NMR: 7.16 (s, 1H, H-3-phenyl), 7.32−7.38 (m, 1H, H-5-benzylidene), 7.47 (d, J=1.8 Hz, 1H, H-3-benzylidene), 7.56 (s, 1H, H-6-phenyl), 8.22 (d, J = 8.47 Hz, 1H, H-6-benzylidene), 8.76 (s, 1H, N=CH). HRMS calculated for $C_{13}H_6Cl_5N$: 350.8943, found 350.4580 (M^+).

(*2,4-Dichlorobenzylidene*)(*2,3,4,5,6-pentafluorophenyl*)*amine* (**6**). Yield: 66%; white solid. IR: 3065, 1532, 1422, 1083, 996, 893, 753. ^{1}H NMR: 7.34−7.40 (m, 1H, H-5-benzylidene), 7.88 (d, J=6 Hz, 1H, H-3-benzylidene), 8.22 (d, J = 6.4 Hz, 1H, H-6-benzylidene), 9.01 (s, 1H, N=CH). HRMS calculated for $C_{13}H_4Cl_2F_5N$: 338.9641, found 338.0766 (M^+).

(*4-Fluorophenyl*)(*3-nitrobenzylidene*)*amine* (**7**) *(13)*. Yield: 68%; yellow solid. Melting point: 90 °C . IR: 3078, 1626, 1526, 1440, 1094. ^{1}H NMR: 7.10−7.13 (m, 1H, H-3-phenyl), 7.21−7.24 (m, 2H, H-4, H-6-phenyl), 7.27−7.29 (m, 1H, H-2-phenyl), 7.63−7.67 (m, 1H, H-5-benzylidene), 8.21−8.23 (m, 1H, H-6-benzylidene), 8.30 (m, 1H, H-4-benzylidene), 8.34 (dd, J = 0.8, 3.6 Hz, 1H, H-2- benzylidene), 8.52 (s, 1H, N=CH). HRMS calculated for $C_{13}H_9FN_2O_2$: 244.0648, found 244.2213 (M^+).

(*3-Fluorophenyl*)(*3-nitrobenzylidene*)*amine* (**8**). Yield: 76%; light yellow solid. Melting point: 62 °C. IR: 3073, 1632, 1585, 1474, 1077. ^{1}H NMR: 6.90−6.93 (m, 1H, H-4-phenyl), 6.96−6.98 (m, 1H, H-5-phenyl), 7.01−7.04 (m, 1H, H-2-phenyl), 7.35 −7.41 (m, 1H, H-6-phenyl), 7.52−7.70 (m, 1H, H-5-benzylidene), 8.34 (m, 1H, H-4-benzylidene), 8.36−8.37 (m, 1H, H-6-benzylidene), 8.53 (s, 1H, N=CH), 8.73−8.75 (m, 1H, H-2-benzylidene). HRMS calculated for $C_{13}H_9FN_2O_2$: 244.0648, found 244.2218 (M^+).

(*2-Fluorophenyl*)(*3-nitrobenzylidene*)*amine* (**9**). Yield: 74%; yellow solid. Melting point: 85 °C. IR: 3085, 1629, 1583, 1456, 1087. ^{1}H NMR: 7.15−7.23 (m, 3H, H-3, H-4, H-5-phenyl), 7.25−7.28 (m, 1H, H-6-phenyl), 8.28−8.31 (m, 1H, H-5-benzylidene), 8.34−8.35 (m, 2H, H-4, H-6-benzylidene), 8.46−8.50 (m, 1H, H-2-benzylidene), 8.64 (s, 1H, N=CH). HRMS calculated for $C_{13}H_9FN_2O_2$: 244.0648, found 244.6591 (M^+).

Furan-2-yl(*3-nitrobenzylidene*)*amine* (**10**). Yield: 78%; yellow solid. Melting point: 67 °C. IR: 3086, 1615, 1531, 1499, 1221. ^{1}H NMR: 6.30 (d, J = 3.04 Hz, 1H, H-3-phenyl), 6.36−6.38 (m, 1H, H-4-phenyl), 7.40−7.50 (m, 1H, H-5-phenyl), 7.56−7.62 (m, 1H, H-5-benzylidene), 8.11 (m, 1H, H-6-benzylidene), 8.26−8.30 (m, 1H, H-4-benzylidene), 8.40 (s, 1H, N=CH), 8.58−8.59 (m, 1H, H-2-benzylidene). HRMS calculated for $C_{11}H_8N_2O_3$: 216.0535, found 216.1938 (M^+).

(*3-Nitrobenzylidene*)(*2,4,5-trichlorophenyl*)*amine* (**11**). Yield: 74%; yellow solid. Melting point: 162 °C. IR: 2955, 1613, 1526, 1456, 1350, 1079, 736. ^{1}H NMR: 6.85 (s, 1H, H-3-phenyl), 7.32 (s, 1H, H-6-phenyl), 7.69−7.79 (m, 2H, Ar-H-5, H-6-benzylidene), 8.24 (m, 1H, H-4-benzylidene), 8.47−8.51 (m, 1H, H-2-benzylidene), 8.72 (s, 1H, N=CH). EI-MS (m/z): 329.1 (M^+ + H), 331.1 (M^+ + 2), 333.1 (M^+ + 4).

(*2,6-Dichlorobenzylidene*)(*4-fluorophenyl*)*amine* (**12**) *(14)*. Yield: 80%; yellow solid. Melting point: 128 °C. IR: 2957, 1634, 1521, 1431, 1375, 1094, 771. ^{1}H NMR: 7.08−7.11 (m, 1H, H-3-benzylidene), 7.13−7.15 (m, 1H, H-5-benzylidene), 7.23−7.25 (m, 2H, H-3, H-5-phenyl), 7.27−7.30 (m, 2H, H-2, H-6-phenyl), 7.38−7.41 (m, 1H, H-4-benzylidene), 8.65 (s, 1H, N=CH). HRMS calculated for $C_{13}H_8Cl_2FN$: 268.0018, found 267.1173 (M^+ − H).

(*2,6-Dichlorobenzylidene*)(*3-fluorophenyl*)*amine* (**13**). Yield: 69%; white solid. Melting point: 72 °C. IR: 2922, 1621, 1584, 1439, 773. ^{1}H NMR: 6.95−6.98 (m, 1H, 4-Ar-H-4- phenyl), 7.01 (m, 1H, H-5-phenyl), 7.04−7.06 (m, 1H, H-2-phenyl), 7.08−7.11 (m, 1H, H-6-phenyl), 7.22−7.26 (m, 1H, H-5-benzylidene), 7.29−7.34 (m, 1H, H-3-benzylidene), 7.36−7.41 (m, 1H, H-4-benzylidene), 8.65 (s, 1H, N=CH). EI-MS (m/z): 268.3 (M^+), 270.2 (M^+ + 2), 272.2 (M^+ + 4).

(*2,6-Dichlorobenzylidene*)(*2-fluorophenyl*)*amine* (**14**). Yield: 83%; white solid. Melting point: 82 °C. IR: 3021, 1612, 1076, 756. ^{1}H NMR: 6.74−6.79 (m, 1H, H-4-phenyl), 6.95−6.97 (m, 1H, H-5-phenyl), 7.13−7.16 (m, 2H, H-3, H-6-phenyl), 7.18−7.25 (m, 1H, H-4-benzylidene), 7.28−7.31 (m, 1H, H-3-benzylidene), 7.38−7.41 (m, 1H, H-5-benzylidene), 8.75 (s, 1H, N=CH). EI-MS (m/z): 268.3 (M^+), 270.2 (M^+ + 2), 272.2 (M^+ + 4).

(*2,6-Dichlorobenzylidene*)*furan-2-ylamine* (**15**). Yield: 77%; yellow liquid. IR: 3078, 1650, 1580, 1431, 1326, 1011, 778. ^{1}H NMR: 6.16−6.28 (m, 1H, H-3-phenyl), 6.34−6.38 (m, 1H, H-4-phenyl), 7.20−7.24 (m, 1H, H-5-phenyl), 7.28−7.31 (m, 1H, H-5-benzylidene), 7.36−7.39 (m, 1H, H-3-benzylidene), 7.42−7.43 (m, 1H, H-4-benzylidene), 8.50 (s, 1H, N=CH). HRMS calculated for $C_{11}H_7Cl_2NO$: 238.9905, found 240.0845 (M^+ + H).

N,N′-Bis(*2,4-dichlorobenzylidene*)*propane-1,3-diamine* (**16**) *(15)*. Yield: 77%; white solid. Melting point: 105 °C. IR: 3074, 2943−2830, 1638, 1467. ^{1}H NMR: 2.12 (m, 2H, 2-CH_2), 3.73−3.78 (m, 4H, 1,3-CH_2), 7.24−7.28 (m, 2H, H-5-Ar), 7.38 (d, J = 2.01 Hz, 2H, H-3-Ar), 7.97 (d, J = 8.49 Hz, 2H, H-6-Ar), 8.60 (s, 2H, N=CH). HRMS calculated for $C_{17}H_{14}Cl_4N_2$: 385.9911, found 385.1175 (M^+).

N,N′-Bis(*2,4-dichlorobenzylidene*)*butane-1,4-diamine* (***17***). Yield: 82%; white solid. Melting point: 120 °C. IR: 2925, 1633, 1583, 1444, 1051. ^{1}H NMR: 1.72−1.83 (m, 4H, 2,3-CH_2), 3.47−3.69 (m, 4H, 1,4-CH_2), 7.24−7.26 (m, 2H, H-5-Ar), 7.31−7.38 (d, J = 1.75 Hz, 2H, H-3-Ar), 7.95 (d, J = 8.49 Hz, 2H, H-6-Ar), 8.63 (s, 2H, N=CH). HRMS calculated for $C_{18}H_{16}Cl_4N_2$: 400.0068, found 400.1453 (M^+).

N,N′-Bis(*2,4-dichlorobenzylidene*)*cyclohexane-1,2-diamine* (***18***). Yield: 84%; white solid. Melting point: 178 °C. IR: 3058, 1638, 1445, 1459. ^{1}H NMR: 1.42−2.00 (m, 8H, 3,4,5,6-CH_2), 3.40−3.50 (m, 1H, N-CH), 3.63−3.65 (m, 1H, N-CH), 7.10- 7.22 (m, 2H, H-5-Ar), 7.28 (d, J = 1.92 Hz, 1H, H-3-Ar), 7.33 (d, J = 1.92 Hz, 1H, H-3′-Ar), 7.86 (d, J = 8.40 Hz, 1H, H-6-Ar), 7.98 (d, J = 8.48 Hz, 1H, H-6′-Ar), 8.52 (s, 1H, N=CH), 8.57 (s, 1H, N=CH). ^{13}C NMR: 23.29, 24.74, 31.30, 33.14, 71.60, 74.19, 127.72, 127.76, 129.67, 129.72, 129.75, 129.98, 132.31, 132.60, 135.75, 135.83, 136.94, 137.02, 155.77, 157.15. HRMS calculated for $C_{20}H_{18}Cl_4N_2$: 426.0224, found 426.2790 (M^+).

N,N′-Bis(*3-nitrobenzylidene*)*propane-1,3-diamine* (***19***). Yield: 80%; white solid. Melting point: 110 °C. IR: 3091, 2925, 2854, 1612, 1528, 1461. ^{1}H NMR: 2.16 (m, 2H, CH_2), 3.81 (t, 4H, 1,3-CH_2), 7.57−7.62 (m, 2H, H-5-Ar), 8.07 (dd, J = 1.80, 2.20 Hz, 2H, H-2-Ar), 8.24−8.25 (m, 2H, H-6-Ar), 8.27−8.28 (m, 2H, H-4-Ar), 8.41 (s, 2H, N=CH). ^{13}C NMR: 32.16, 59.42 (2C), 123.14 (2C), 125.34 (2C), 130.00 (2C), 133.94 (2C), 138.94 (2C), 149.02 (2C), 159.09 (2C). HRMS calculated for $C_{17}H_{16}N_4O_4$: 340.1172, found 340.3335 (M^+).

N,N′-Bis(*3-nitrobenzylidene*)*butane-1,4-diamine* (***20***) *(16)*. Yield: 81%; white solid. Melting point: 112 °C. IR: 3092, 2945−2850, 1612, 1523, 1476. ^{1}H NMR: 1.79−1.84 (m, 4H, 2,3-CH_2), 3.69−3.72 (m, 4H, 1,4-CH_2), 8.03−8.05 (m, 2H, H-5-Ar), 8.13−8.17 (m, 2H, H-6-Ar), 8.25 (dd, J = 1.2, 3.6 Hz, 2H, H-2-Ar), 8.34 (s, 2H, N=CH), 8.53−8.54 (m, 2H, H-4-Ar). HRMS calculated for $C_{18}H_{18}N_4O_4$: 354.1328, found 354.6820 (M^+).

N,N′-Bis(*3-nitrobenzylidene*)*cyclohexane-1,2-diamine* (***21***) *(17)*. Yield: 74%; white solid. Melting point: 112 °C. IR: 3024, 2934, 2867, 1613, 1527, 1442. ^{1}H NMR: 1.46−1.52 (m, 4H, 4,5-CH_2), 1.74−1.89 (m, 4H, 3,6-CH_2), 3.49 (m, 2H, N-CH), 7.87−7.89 (m, 2H, H-5-Ar), 8.12−8.15 (m, 2H, H-6-Ar), 8.16 (dd, J = 1.20, 3.60 Hz, 2H, H-2-Ar), 8.25 (s, 2H, N=CH), 8.37−8.51 (m, 2H, H-4-Ar). HRMS calculated for $C_{20}H_{20}N_4O_4$: 380.1485, found 380.3963 (M^+).

N,N′-Bis(*2,6-dichlorobenzylidene*)*butane-1,4-diamine* (***22***). Yield: 79%; white solid. Melting point: 120 °C. IR: 2927, 2866, 1645, 1582, 1428, 1379, 1033. ^{1}H NMR: 1.67−1.91 (m, 4H, 2,3-CH_2), 3.70−3.78 (m, 4H, 1,4-CH_2), 7.17−7.23 (m, 2H, H−Ar), 7.31−739 (m, 4H, H-3, H-5-Ar), 8.44 (s, 2H, N=CH). ^{13}C NMR: 26.92 (2C), 60.69 (2C), 126.63 (2C), 127.27 (2C), 128.82 (2C), 132.067 (2C), 133.31 (2C), 135.71 (2C), 155.47 (2C). HRMS calculated for $C_{18}H_{16}Cl_4N_2$: 400.0068, found 400.1443 (M^+).

N,N′-Bis(*2,6-dichlorobenzylidene*)*cyclohexane-1,2-diamine* (***23***). Yield: 67%; white solid. Melting point: 130 °C. IR: 3054, 2928−2801, 1630, 1581, 1430, 1337, 1093, 726. ^{1}H NMR: 1.51−1.59 (m, 4H, 4,5-CH_2), 1.67−1.89 (m, 4H, 3,6-CH_2), 3.58−3.64 (m, 2H, N-CH), 7.12−7.15 (m, 2H, H-4-Ar), 7.26−7.28 (m, 4H, H-3, H-5-Ar), 8.49 (s, 2H, N=CH). EI-MS (m/z): 427.1 ($M^+ + H$), 429.0 ($M^+ + 2$), 431.0 ($M^+ + 4$), 433.0 ($M^+ + 6$).

***In Vitro* Antifungal Activity.** The above-synthesized compounds were tested for their ability to inhibit soil borne pathogenic fungi against the standard fungicide hexaconazole. The concentrations of the latter were those recommended by the manufacturer. The fungicidal activity of synthesized compounds was evaluated at various concentrations by the poisoned food technique using PDA media. The readymade PDA medium (39 g) was suspended in distilled water (1000 mL) and heated to boiling until completely dissolved. The medium and Petri dishes were autoclaved at 120 °C for 30 min. These compounds were tested at concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.90 μg/mL. A stock solution of 1000 μg/mL was prepared in DMSO, which was further diluted with DMSO to give the required concentrations. DMSO (1 mL) was used as a control. These solutions were added to the media (65 mL, temperature 40 °C) in conical flasks to obtain the desired concentrations of the test compounds in the media. The medium was poured into a set of two Petri dishes (90 cm in diameter) under aseptic conditions in a laminar flow hood. The plates were kept under UV light in the laminar flow chamber for solidification of the media. After solidification, a 5 mm (diameter) mycelia plug cut from the actively growing front of a 2 week old colony of the desired pathogenic fungus was then placed with the inoculum's side down in the center of each treatment plate, aseptically. Treated Petri dishes were then incubated at 28 °C until the fungal growth was almost complete in the control plates. All experiments were in quadruplicate for each treatment against each fungus.

Recording of Observations. The mycelial growth of fungus (cm) in both treated (T) and control (C) Petri dishes was measured diametrically. The mean and standard errors were calculated from the four replicates of each treatment, and the percentage inhibition of growth (I) was calculated using the following formula:

$$\text{inhibition (\% I)} : (C - T) \times 100/C$$

Calculation of ED_{50} Values. For calculation of ED_{50} values (effective dose required for 50% inhibition of growth), the percent inhibition was converted to corrected percent inhibition by using Abbott's formula:

$$\text{corrected inhibition (\%)} : (I - \text{CF}) \times 100/(100 - \text{CF})$$

where CF is the correction factor obtained by the equation

$$\text{correction factor (CF)} : (9 - C) \times 100/C$$

where 9 is the diameter of the Petri dish in cm and C is the diameter of growth of the fungus in control plates. From the concentration (μg/mL) and corresponding corrected percentage inhibition data of each compound, the ED_{50} (μg/mL) value was calculated statistically by Probit analysis with the help of Probit package of MSTATC software using a personal computer. ED_{50} values were calculated (effective dose for 50% inhibition) for inhibition of growth using the Basic LD_{50} program version 1.1.

Evaluation of Nitrification Inhibition. *Soil*. The soil for the *in vitro* incubation experiments was collected from the farm of the Institute. Composite soil sample was collected in bulk from the cultivated fields of known history from a depth of 0−15 cm following standard sampling procedure. The physical and chemical characteristics of the soil were as follows: sand 60.8%, clay 20.5% and silt 18.7% pH 7.9 (soil: water 1:2.5); EC at 25 °C 0.35 dS m^{-1}; organic carbon 0.50%; available N 55.72 mg/kg soil, nitrate-N 12.9 mg/kg soil, nitrite-N (traces) and ammonium-N 5.6 mg/kg soil. It was air-dried at room temperature, ground and passed through 2 mm sieve. The soil was thoroughly mixed before use.

Experiment. The experiments were laid following completely randomized design (CRD) with three replicates. The test chemicals and reference inhibitor were tested at 5% dose of applied urea-N along with urea alone control. The samples were incubated in 100 mL capacity plastic beakers (50 g of air-dried soil was taken per beaker). Calculated amount of the test chemical (0.5 mg for 5% dose of applied urea-N, respectively) in acetone was added to each beaker and mixed thoroughly. In all the treatments including control, the same volume of acetone was added. After thorough mixing, 10 mg of urea-N (200 mg of urea-N per kg of soil) in aqueous solution was added and mixed thoroughly. Distilled water was added to each beaker for maintaining the moisture at 50% water holding capacity of the soil. The controls were similarly processed with urea alone at the 200 mg/kg urea-N level without adding any test/reference inhibitor. All the beakers were accurately weighed, labeled and kept at 28 ± 1 °C and 98% relative humidity in an incubator. Soil moisture was maintained by adding distilled water every alternate day after taking the difference of weight if necessary.

Sampling and Estimation of Ammonium, Nitrite and Nitrate-N from the Soil Samples. 5 g of soil samples were withdrawn on the 21st day of incubation (*21*). Ammonium nitrite and nitrate-N were extracted in 50 mL aqueous sodium sulfate solution (1 M). The soil with extracting solution was shaken for an hour on a reciprocal shaker and filtered. ammonium-N, nitrite-N and nitrate-N were estimated by Indophenol blue, sulfanilic acid and phenol disulfonic acid methods (*18*) respectively. The contents of ammonium, nitrite and nitrate-N were obtained from the standard curves and expressed in mg/kg. The nitrification rate (NR) and percent nitrification inhibition (NI) was calculated using Sahrawat's formulas (*20*).

Table 1. Antifungal Activity of Schiff Bases against *Sclerotium rolfsii* (SR) and *Rhizoctonia bataticola* (RB) *in Vitro*

			ED_{50}[a] (μg/mL), pathogenic fungi		chi square for heterogeneity[b]	
compound no.	Ar	R	SR	RB	SR	RB
1	2,4-dichlorophenyl	4-fluorophenyl	37.44	21.82	2.36	1.49
2	2,4-dichlorophenyl	3-fluorophenyl	548.25	600.38	3.98	1.26
3	2,4-dichlorophenyl	2-fluorophenyl	63.42	15.56	1.68	4.19
4	2,4-dichlorophenyl	2-furyl	35.21	23.15	1.56	2.27
5	2,4-dichlorophenyl	2,4,5-trichlorophenyl	24.37	3.05	1.04	5.01
7	3-nitrophenyl	4-fluorophenyl	164.06	190.25	1.60	2.48
8	3-nitrophenyl	3-fluorophenyl	175.25	180.00	4.63	5.20
9	3-nitrophenyl	2-fluorophenyl	160.25	112.87	3.19	1.46
10	3-nitrophenyl	2-furyl	201.07	17.12	3.95	2.17
11	3-nitrophenyl	2,4,5-trichlorophenyl	21.71	6.17	3.27	3.88
12	2,6-dichlorophenyll	4-fluorophenyl	107.95	123.17	5.02	1.99
13	2,6-dichlorophenyl	3-fluorophenyl	86.95	53.99	3.13	4.92
14	2,6-dichlorophenyl	2-fluorophenyl	45.62	44.95	4.961	1.687
15	2,6-dichlorophenyl	2-furyl	78.29	45.66	3.73	4.28
16	2,4-dichlorophenyl	$-(CH_2)_3-$	27.14	17.75	1.31	2.77
17	2,4-dichlorophenyl	$-(CH_2)_4-$	178.52	100.25	1.89	3.23
18	2,4-dichlorophenyl	cyclohexyl-1,2-	34.22	18.28	1.40	1.39
19	3-nitrophenyl	$-(CH_2)_3-$	na	na	na	na
20	3-nitrophenyl	$-(CH_2)_4-$	na	na	na	na
21	3-nitrophenyl	cyclohexyl-1,2-	na	na	na	na
22	2,6-dichlorophenyl	$-(CH_2)_4-$	22.05	12.42	1.49	1.66
23	2,6-dichlorophenyl	cyclohexyl-1,2-	39.53	124.07	1.48	2.50
24	hexaconazole (standard fungicide)		2.30	1.70	1.50	1.02

[a] Mean percentage inhibition, used for ED_{50} calculation, is an average of four replicates and its standard deviation (±) ranged from ±0.2 to ±2.3. [b] Chi square for heterogeneity (tabular value at 0.05 level) = 5.991 (degrees of freedom = 3); na, not active.

Statistical Treatment of the Data. The experimental data were statistically analyzed following the procedure laid out by Gomez and Gomez (*21*). The analysis of variance was computed using Statistical Package for Social Services (SPSS version 10.0), and treatment means were compared by using Duncan's Multiple Range Test (DMRT) at 5% levels.

RESULTS AND DISCUSSION

Synthesis. *Substituted Imines*. Substituted imines were synthesized (**Figure 1**) by the reaction of equimolar amounts of aldehydes, namely, 2,4-dichlorobenzaldehyde, 3-nitrobenzaldehyde, 2,6-dichlorobenzaldehyde, and amines such as 2/3/4-fluoroaniline, 2-furfurylamine, 2,4,5-trichloroaniline, 1,2,3,4,5-pentafluoroaniline in dry methanol to afford substituted imines. All the imines (**1**−**15**) were characterized on the basis of spectral data and microanalysis.

Diaryl Methylene Diimines. Reaction of 2 equiv of aforesaid aromatic aldehydes with 1 equiv of 1,3-propyl/1,4-butyl/1,2-cyclohexyl diamines separately in methanol resulted in diaryl methylene diimines (**16**−**23**) in excellent yields (**Figure 2**), which were characterized using spectral techniques.

The ^{1}H NMR spectra of Schiff bases (**1**−**23**) exhibited a characteristic 1H- singlet (*HC*═N) in the range of δ 8.40−9.01 ppm, respectively. The IR spectra of Schiff bases (**1**−**23**) showed a characteristic peak in the range of 1613−1650 cm^{-1} for C═N absorption.

***In Vitro* Antifungal Activity.** All the Schiff bases (**1**−**23**) were screened for the fungicidal activity against *S. rolfsii* and *R. bataticola* by the poisoned food technique, and their ED_{50} values are reported in **Table 1**. Maximum antifungal activity was observed with compound **5** (ED_{50} = 3.05 and 24.37 μg/mL against *R. bataticola* and *S. rolfsii* respectively) and compound **11** (ED_{50} = 6.17 and 21.70 μg/mL against *R. bataticola* and *S. rolfsii*, respectively). The antifungal activity of compound **5** (**Table 1**) against *R. bataticola* was comparable to the standard fungicide hexaconzole (ED_{50} = 1.70 μg/mL). Besides these, compounds **1**, **3**, **4**, **16** and **18** were also found effective against *R. bataticola* (ED_{50} = 21.82, 15.56, 23.15, 17.75, and 18.29 μg/mL). It was observed that compounds containing chlorine atoms (**Figures 3** and **4**) showed better antifungal activity than compounds containing nitro substituents (**Figure 5**) except in the case of compound **11**. It was also observed that if chlorine atom is present at the 2 and 4 positions, the antifungal activity is more as compared to the 2 and 6 positions (**Figures 3** and **4**). The presence of the 2-furyl group also increased the activity. Among diimines, compound **22** (ED_{50} = 12 μg/mL and 22 μg/mL against *R. bataticola and S. rolfsii* respectively) showed maximum antifungal activity whereas compound **23** was found effective only against *S. rolfsii* (ED_{50} = 39.53 μg/mL).

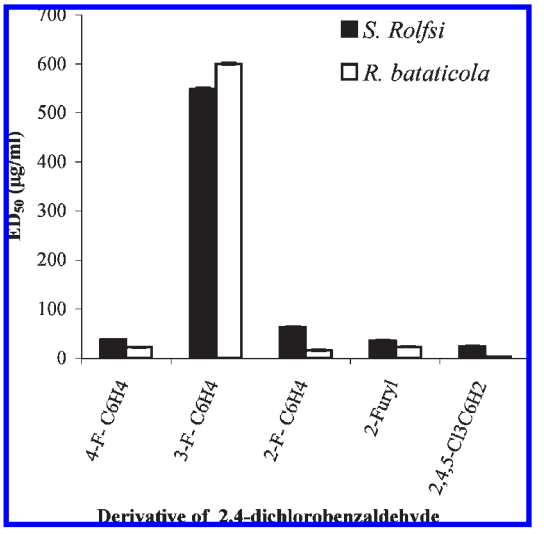

Figure 3. Fungicidal activity of potent 2,4-dichlorobenzylidene aryl amines against *S. Rolfsii* and *R. Bataticola in vitro* (error bars shows standard deviation).

In general, monoimines derivatives showed better antifungal activity as compared to diimines. Antifungal activity was pronounced against *R. bataticola* than *S. rolfsii*.

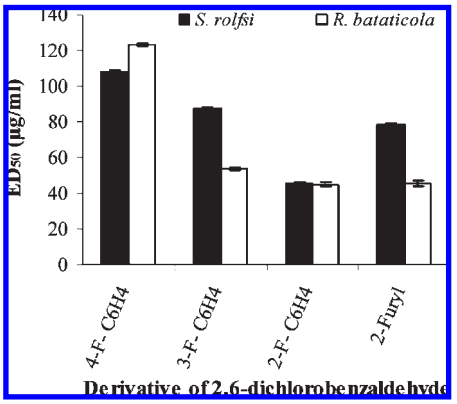

Figure 4. Fungicidal activity of potent 2,6-dichlorobenzylidene aryl amines against *S. Rolfsii* and *R. Bataticola in vitro* (error bars shows standard deviation).

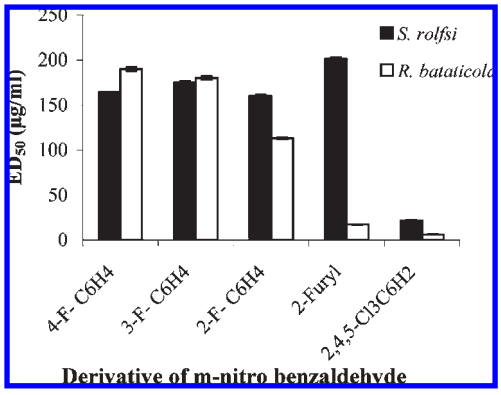

Figure 5. Fungicidal activity of potent 3-nitrobenzylidene aryl amines against *S. Rolfsii* and *R. Bataticola in vitro* (error bars shows standard deviation).

Nitrification Inhibitory Activity. Results obtained in the *in vitro* soil incubation study are reported in **Table 2**.

Ammonium-N Content. Significantly higher amount of ammonium-N were observed in most of the test compounds (24−104 mg/kg) as compared to urea alone (24 mg/kg). Nitrapyrin, 140 mg/kg is most effective in retention of ammonium-N. Compound **12** showed maximum ammonium-N retention with value 104 mg/kg and significantly superior to others as evidenced from DMRT values. The next in performance were compounds **13**, **3**, **17**, **5**, **22**, **18**, **21**, **16**, **20** with 67−93 mg/kg ammonium-N. The rest of the compounds lay in the range of 24−64 mg/kg ammonium-N. From the overall performance it was found that the diimines (**16**−**23**) showed moderate ammonium-N content (64−70 mg/kg) while monoimines showed low to high ammonium-N content (24−104 mg/kg). Among monoimines 2-furyl as R resulted in the decrease in the retention of ammonium-N within the group having the same Ar.

Nitrite-N. The nitrite-N content was 0−5 mg/kg in most of the samples treated with monoimines except compounds **5** and **11**, and all were statistically at par with each other as supported by DMRT values. Introduction of 2,4,5-trichlorophenyl resulted in the accumulation of nitrite as the nitrite-N content with compound **5** and **11** were 36 and 24 mg/kg. This suggested their nonselective and undesirable inhibition of nitrite to nitrate oxidation. These compounds were effective in inhibiting the first step as evidenced from 72 and 62 mg/kg ammonium-N. It was also observed that most of the diimines showed higher amount of nitrite-N content (36−44 mg/kg) except compounds **17**, **22** and **23**.

Nitrate-N. Significantly less nitrate-N content was observed in all the test chemicals (3−22 mg/kg) except compounds **6** (159 mg/kg), **4** (109 mg/kg), **10** (106 mg/kg), **9** (61 mg/kg) and **15** (52 mg/kg) compared to urea alone (118 mg/kg). Among diimines all compounds were active having nitrate-N in the range of (10−22 mg/kg). Compound **23** was most active (9 mg/kg) followed by **18** and **22** (10 mg/kg). All these compounds were statistically similar

Table 2. Effect of Schiff Bases on Nitrogen Dynamics in Soil

compound no.	Ar	R	ammonium-N (mg/kg)	nitrite-N (mg/kg)	nitrate-N (mg/kg)	NR[a] (%)	NI[a] (%)
1	2,4-dichlorophenyl	4-fluorophenyl	55 j	5 d	13 fghi	25 i	70 d
2	2,4-dichlorophenyl	3-fluorophenyl	49 k	4 d	13 ghij	26 i	69 d
3	2,4-dichlorophenyl	2-fluorophenyl	90 c	0 d	3 ij	4 k	96 a
4	2,4-dichlorophenyl	2-furyl	24 mn	1 d	109 c	82 a	2 L k
5	2,4-dichlorophenyl	2,4,5-trichlorophenyl	72 e	36 b	10 ghij	39 gh	54 ef
6	2,4-dichlorophenyl	2,3,4,5,6-pentafluorophenyl	33 m	1 d	159 a	83 a	1 lk
7	3-nitrophenyl	4-fluorophenyl	61 hij	0 d	4 ij	7 k	92 a
8	3-nitrophenyl	3-fluorophenyl	62 ghij	0 d	16 fg	21 ij	75 cd
9	3-nitrophenyl	2-fluorophenyl	38 lm	4 d	61 d	63 c	25 j
10	3-nitrophenyl	2-furyl	24 n	0 d	106 c	82 a	2 lk
11	3-nitrophenyl	2,4,5-trichlorophenyl	60 ij	24 c	7 hij	34 f	59 e
12	2,6-dichlorophenyl	4-fluorophenyl	104 b	0 d	5 hij	5 k	95 a
13	2,6-dichlorophenyl	3-fluorophenyl	93 c	3 d	4 ij	7 k	91 a
14	2,6-dichlorophenyl	2-fluorophenyl	48 k	5 d	10 ghij	24 1i	72 d
15	2,6-dichlorophenyl	2-furyl	44 kl	2 d	52 e	55 d	34 i
16	2,4-dichlorophenyl	$-(CH_2)_3-$	68 efgh	40 ab	17 fg	46 ef	45 gh
17	2,4-dichlorophenyl	$-(CH_2)_4-$	80 d	0 d	14 fgh	15 jh	82 bc
18	2,4-dichlorophenyl	cyclohexyl-1,2-	69 efg	39 b	10 ghij	41 fg	50 fg
19	3-nitrophenyl	$-(CH_2)_3-$	64 fghi	44 a	18 fg	49 e	41 h
20	3-nitrophenyl	$-(CH_2)_4-$	67 efghi	36 b	17 fg	44 efg	47 fgh
21	3-nitrophenyl	cyclohexyl-1,2-	69 efg	41 ab	22 f	48 e	43 h
22	2,6-dichlorophenyl	cyclohexyl-1,2-	56 j	0 d	9 ghij	14 h	84 b
23	2,6-dichlorophenyl	$-(CH_2)_4-$	70 ef	1d	10 ghij	14 h	83 b
urea			24 n	0 d	118 b	83 a	
nitrapyrin			140 a	0 d	6 hij	5 h	95 a

[a] NR, nitrification rate; NI, nitrification inhibition; means are the average of three replicates. Means followed by a common letter are not significantly different at the 5% level based on Duncan's Multiple Range Test (DMRT). Standard deviation (±) with mean of three replicates ranged from ±0.35 to ±4.12.

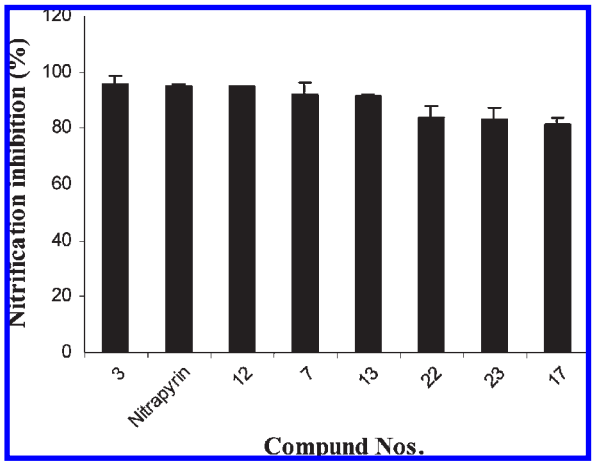

Figure 6. Effect of potent Schiff bases and nitrapyrin on nitrification inhibition (%) (error bars shows standard deviation).

with each other and with nitrapyrin (6 mg/kg) as they fall in the same group in DMRT. Among monoimines, compounds with 2-furyl/2,3,4,5,6-pentafluorophenyl as R were not effective in lowering the nitrate-N content irrespective of Ar. Compounds with fluorophenyl group as R were best performers with 3−16 mg/kg nitrate-N similar to that of nitrapyrin. Only one compound **9** with 2-fluoro phenyl as R and 3-nitrophenyl as Ar was an exception to the above statement.

Nitrification Inhibition (NI). All the Schiff bases showed a wide range (1−96% NI) effect on nitrification inhibition (**Table 2**). Compounds **3**, **7**, **12**, **13** were the most active ones among all the test compounds showing NI in the range (91−96%) statistically similar to nitrapyrin (95%, **Figure 6**). The next in performance were compounds **22** (84%), **23** (83%), **17** (82%), **8** (75%), **14** (72%), **1** (70%) and **2** (69%). The rest of the compounds showed less than 60% NI. The fluorine containing compounds emerged as potential NI (72−96%) except compounds **6** (1%) and **9** (5%). Compounds with 2-furyl group as R in the test molecules led to a decrease in the activity. This is a quite unusual behavior after introduction of furyl group in the test molecules as furan compounds are already known as potent nitrification inhibitors (*21*). The compounds with 2,6-dichlorophenyl group as Ar irrespective of R group inhibited nitrification effectively (34 and 71−96%). Its combination with 2-furyl as R resulted in an increase in NI from 2 to 34%.

Cumulative Effect of Schiff Bases. Antifungal and nitrification inhibitory data of synthesized Schiff bases revealed that monoimine derivatives exhibit the best activity. But there is no single molecule which can be exploited for both activities. An overview of activity data with respect to diimine derivatives revealed that compound **22** emerged as potent fungicide and nitrification inhibitor. Simple cost-effective novel fungicidal and nitrification inhibitory molecules were developed during the study. As a sequel the potent compounds are planned to be taken up for pot and field experiments.

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