



Plant Pathology Journal

ISSN 1812-5387

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

***In vitro* Antifungal Activity of Novel Picolinamides against Soil Borne Fungi and Structure Activity Relationship**

Aditi Kundu and Supradip Saha

Division of Agricultural Chemicals, Indian Agricultural Research Institute, Delhi, 110012, India

Abstract: We investigated the antifungal potentiality of multifunctional novel picolinamide derivatives against various phytopathogens. Picolinic acid is a microbial secondary metabolite reported to possess wide biological potential. Picolinic acid was esterified, condensed with hydrazine hydrate and subsequent refluxing with various substituted aromatic aldehydes to synthesize sixteen novel substituted picolinamides. Synthesized novel compounds were characterized by various physico-spectral techniques. Structure antifungal activity relationship of the synthesized molecules was predicted by evaluating individual derivatives. Picolinamide derivatives were found to possess significant antifungal activity against the wide range of soil borne pathogens. Chloro substituted picolinamide derivatives exhibited maximum antifungal activity against *R. solani* (ED_{50} 29.08 $\mu\text{g mL}^{-1}$) followed by *A. alternata* (ED_{50} 33.90 $\mu\text{g mL}^{-1}$). Antifungal bioassay results testify that these compounds can be of interest in search for new fungicides.

Key words: Picolinic acid, antifungal activity, picolinamide, structure-activity relationship

INTRODUCTION

Research on novel synthesis of amides has received considerable attention in recent years. Imines are the compounds containing a carbon-nitrogen double bond which imparts both potential chemical and biological activity (Dhar and Taploo, 1982; Yang *et al.*, 2002). Imines are known for their wide range of therapeutic application such as antimicrobial, antimycobial, anti-inflammatory, antiviral (Jarrahpour *et al.*, 2007), antioxidant activity (Tang and Liu, 2007; Borawska *et al.*, 2008), nitrification inhibitor (Aggarwal *et al.*, 2009), anticancer (Jesmin *et al.*, 2010) and anticonvulsant activity. It is an important precursor of several biologically versatile heterocyclic compounds like benzoxazines, pyrazoles, thiazolidinones etc. (Dangi *et al.*, 2011).

Butyl derivative of picolinic acid, known as fusaric acid is an important secondary metabolite of fungi *Fusarium* sp. and also reported to possess significant antimicrobial activity. Chloro substituted derivatives of picolinic acid exhibited significant plant growth regulatory activity (Hamaker *et al.*, 1963). Ethyl picolinate and picolinamide also inhibits sporulation process of microorganisms by undergoing hydrolysis intracellularly (Upreti *et al.*, 1969). Picolinic acid amides and hydroxy derivatives are powerful anthelmintic (Newell *et al.*, 1984) and antimicrobial (Hoveyda *et al.*, 1998) agent. Picolinic acid is also a metabolite of fungi (e.g. *Fusarium* spp.), known as potential phenoloxidase inhibitor (Dowd, 1999). Picolinic acid is an important metal ion chelator

(Dazzi *et al.*, 2001) which potentiates macrophage antimycobacterial activity (Koczon *et al.*, 2005; Cai *et al.*, 2006; Musk Jr. and Hergenrother, 2008) activity. Picolinic acid is a member of the pyridine family with a carboxyl side chain at the 2-position (Coggan *et al.*, 2009). Recently, picolinic acid derivatives are evaluated as antitubercular agent (Lingala *et al.*, 2011). Versatile potentiality of picolinic acids has given zeal to design and synthesize the novel amides of picolinic acid with the aim to develop antifungal agent. A perusal of the literature revealed that there is no report of synthesis and biological activity of picolinamide derivatives. Hence, the purpose of the present research is to synthesize novel picolinamide derivatives to investigate their antifungal toxicity.

MATERIALS AND METHODS

Analytical instruments: Melting points were recorded on an Electro thermal type 9100 melting point apparatus and are not corrected. ^1H NMR spectra were recorded on Bruker Avance (400 MHz) instrument. Chemical shifts are reported in δ (ppm) units with respect to TMS as internal standard and coupling constants (J) are reported in Hz units. Mass spectra were recorded on a mass spectrometer at 70 eV, ESI-MS (electron spray ionization mass spectrometry) was performed with a Quattro triple-quadrupole mass spectrometer (Thermo Finnigan MAT Incos 50, USA). The elemental analysis was done on a Eurovector Elemental analyzer 3000 using

sulphanilamide as standard with linear calibration. Reagents used for the experiment were commercial grade procured from Sigma® (USA) and Merk® India Ltd. (Navi Mumbai, India). Potato Dextrose Agar (PDA) was procured from Hi Media® Laboratories (Mumbai, India). Reactions were monitored by Thin Layer Chromatography (TLC) on pre-coated silica gel F₂₅₄ plates from Merck® and visualized either by exposure to UV light or 10% H₂SO₄ solution. Laboratory grade reagents and solvents were locally procured.

Synthesis of picolinamide derivatives: Picolinic acid (0.01 mol) was refluxed with sulphuric acid (50 mL) and absolute alcohol (115 mL) for 6 h and the mixture was cooled to the room temperature and poured on to the crushed ice. The mixture was then made strongly alkaline by the addition of ammonia solution. The resulting mixture was extracted with diethyl ether. Solvent was then distilled off and the resultant liquid ester (2) was recovered. Ethyl picolinate (0.01 mol) was condensed with hydrazine hydrate for 6 h by maintaining the reaction temperature at 0°C to obtain solid picolinic acid hydrazide (3). The resultant hydrazide was re-crystallized from warm ethanol. Picolinic acid hydrazide (0.01 mol) was further refluxed with various substituted aromatic aldehydes (0.02 mol) in the presence of sulphuric acid for 5 h. The reaction mixture was then poured into the crushed ice; the resultant solid was washed with distilled water, dried and re-crystallized with ethanol to obtain picolinamide derivatives (4a-p).

Under microwave assisted synthesis, picolinic acid hydrazide was prepared from ethyl picolinate (0.01 mol) and hydrazine hydrate for 3 min of microwave irradiation. The synthesized hydrazide and various substituted aromatic aldehydes were taken separately in 50 mL flask. The reaction mixture was irradiated inside a microwave oven along with distilled water as dummy. The microwave irradiation was carried out in different runs of 10 sec and the reaction was monitored by TLC. The reaction was complete within 3 min. The reaction mixture was cooled to room temperature and yellow solid separated, which on re-crystallization with petroleum ether: CHCl₃ (9:1, v/v) mixture gave bright yellow crystals having sharp melting points.

Fungicidal bioassay: Plant pathogenic fungi, namely, *Rhizoctonia solani* ITCC 2775, *Alternaria alternata* ITCC 5501, *Sclerotium rolsii* ITCC 5512, *Fusarium oxysporum* ITCC 1053 and *Macrophomina phaseolina* ITCC 3134 were purchased from the Indian Type Culture Collection (ITCC), Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi, India. Pathogenic fungi

were maintained on Potato Dextrose Agar (PDA) at 25°C and were sub-cultured on PDA Petri dishes for 5-6 days at 28°C prior to use as inoculums.

Picolinamide derivatives were tested for their antifungal activity against pathogenic fungi namely, *R. solani*, *A. alternata*, *S. rolsii*, *F. oxysporum* and *M. phaseolina*, at various concentrations by the poisoned food technique using Potato Dextrose Agar media (PDA media) against the standard fungicide Bavistin®. The ready-made 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. A stock solution of 1000 µg mL⁻¹ of the test compound was prepared which was further diluted with acetone to give the required concentrations of 500, 250, 125, 62.5 µg mL⁻¹. Only acetone (1 mL) was used in the control plates instead of test compounds. These solutions were added to the media (65 mL) contained in conical flasks to obtain the desired concentrations of the test compound in the media. The medium was poured into a set of two Petri dishes (90 mm diameter) under aseptic conditions under laminar flow. After solidification, a 5 mm mycelial disk cut from the actively growing front of a 2 weeks old colony of the desired pathogenic fungus was then placed with the inoculums side down in the centre of each treated Petri dish, 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. The mycelial growth of fungus (cm) in both treated (T) and control (C) Petri dishes were measured diametrically. The mean and standard deviation were calculated from the four replicates of each treatment and the percentage inhibition of growth (% I) was calculated using the following equation:

$$I(\%) = \frac{C - T}{C} \times 100$$

For calculation of ED₅₀ values (effective dose required for 50% inhibition of growth), the percent inhibition was converted to corrected percent inhibition by using equation:

$$\text{Corrected inhibition (\%)} = \frac{(\% I - CF)}{(100 - CF)} \times 100$$

where, CF is the correction factor obtained by the equation:

$$\text{Correction factor (CF)} = \frac{(90 - C)}{C} \times 100$$

where, 90 is the diameter of the petri dish in mm and C is the diameter of growth of the fungus in control plates.

From the concentration ($\mu\text{g mL}^{-1}$) and corresponding corrected percent inhibition data of each compound, the ED_{50} ($\mu\text{g mL}^{-1}$) value was calculated statistically by robit analysis with the help of Probit Package of MSTATC software using a personal computer. ED_{50} values (effective dose required for 50% inhibition $\mu\text{g mL}^{-1}$) were calculated using the Basic LD_{50} programme version 1.1.

Statistical analysis: The experimental data were analysed statistically and 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% significance level.

RESULTS

Characterization of synthesized picolinamide derivatives:

Picolinic acid ester (2) was prepared by refluxing picolinic acid in presence of sulphuric acid and absolute ethyl alcohol. Ethyl picolinate was condensed with hydrazine hydrate to synthesize solid picolinic acid hydrazide (3), which was again refluxed with various substituted aromatic aldehydes to form a series of novel picolinamide derivatives (4a-p) (Fig. 1).

N-phenylimino-picolinamide (4a): Light yellow coloured solid, Yield 80.3%, mp 165-169°C, R_f : 0.64 (hexane:chloroform, 9:1). IR (nujol) cm^{-1} : 2926 (C-H), 1620 (C = N), 1712 (C = O), 3380 (N-H), 1600, 1590, 1500 and 1460 (aromatic ring); $^1\text{HNMR}$ (400 MHz, CDCl_3 , ppm): δ 5.92 (s, 2H, -NCH₂), 3.58 (s, 1H, -NH), 6.65-6.81 (m, 4H, pyridine), 7.77-8.15 (m, 5H, phenyl protons); ESI-MS m/z : 224.7 (M^+), 226.2 ($M^+ + 2$); Anal. Calcd. for: $\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}$: C, 69.33; H, 4.89; N, 18.67. Found C, 69.52; H, 4.85; N, 18.81.

N-phenyl-(2-chloro)-imino-picolinamide (4b): Light yellow coloured solid, Yield 78.9%, mp 181-184°C, R_f : 0.53 (hexane:chloroform, 9:1). IR (nujol) cm^{-1} : 2976 (C-H), 1622 (C = N), 1710 (C = O), 3375 (N-H), 1604, 1588, 1508 and 1460 (aromatic ring); $^1\text{HNMR}$ (400 MHz, CDCl_3 , ppm): δ 5.79 (s, 2H, -NCH₂), 3.54 (s, 1H, -NH), 6.61-6.85 (m, 4H, pyridine), 7.69-8.13 (m, 4H, phenyl protons); ESI-MS m/z : 258.0 (M^+), 260.5 ($M^+ + 2$); Anal. Calcd. for: $\text{C}_{13}\text{H}_{10}\text{N}_3\text{OCl}$: C, 60.23; H, 3.86; N, 16.22. Found C, 60.18; H, 3.85; N, 16.28.

N-phenyl-(3-chloro)-imino-picolinamide (4c): Light yellow coloured solid, Yield 84.2%, mp 175-178°C, R_f : 0.57 (hexane:chloroform, 9:1). IR (nujol) cm^{-1} : 2920 (C-H), 1618 (C = N), 1715 (C = O), 3378 (N-H), 1606, 1590, 1570 and

1498 (aromatic ring); $^1\text{HNMR}$ (400 MHz, CDCl_3 , ppm): δ 5.93 (s, 2H, -NCH₂), 3.56 (s, 1H, -NH), 6.54-6.71 (m, 4H, pyridine), 7.71-8.19 (m, 4H, phenyl protons); ESI-MS m/z : 258.4 (M^+), 260.1 ($M^+ + 2$), 262.0 ($M^+ + 4$); Anal. Calcd. for: $\text{C}_{13}\text{H}_{10}\text{N}_3\text{OCl}$: C, 60.23; H, 3.86; N, 16.22. Found C, 60.27; H, 3.80; N, 16.20.

N-phenyl-(4-chloro)-imino-picolinamide (4d): Light yellow coloured solid, Yield 85.5%, mp 179-181°C, R_f : 0.61 (hexane:chloroform, 9:1). IR (nujol) cm^{-1} : 2920 (C-H), 1612 (C = N), 1728 (C = O), 3336 (N-H), 1580, 1576, 1520 and 1506 (aromatic ring); $^1\text{HNMR}$ (400 MHz, CDCl_3 , ppm): δ 5.89 (s, 2H, -NCH₂), 3.63 (s, 1H, -NH), 6.76-6.89 (m, 4H, pyridine), 7.34-7.95 (m, 4H, phenyl protons); ESI-MS m/z : 258.3 (M^+), 260.7 ($M^+ + 2$); Anal. Calcd. for: $\text{C}_{13}\text{H}_{10}\text{N}_3\text{OCl}$: C, 60.23; H, 3.86; N, 16.22. Found C, 60.27; 3.75; N, 16.29.

N-phenyl-(4-fluoro)-imino-picolinamide (4e): Yellow coloured solid, Yield 87.1%, mp 156-158°C, R_f : 0.67 (hexane:chloroform, 9:1). IR (nujol) cm^{-1} : 2920 (C-H), 1620 (C = N), 1718 (C = O), 3380 (N-H), 1600, 1510, 1480 and 1450 (aromatic ring); $^1\text{HNMR}$ (400 MHz, CDCl_3 , ppm): δ 5.88 (s, 2H, -NCH₂), 3.60 (s, 1H, -NH), 6.68-6.86 (m, 4H, pyridine), 7.64-8.10 (m, 4H, phenyl protons); ESI-MS m/z : 242.0 (M^+), 244.4 ($M^+ + 2$); Anal. Calcd. for: $\text{C}_{13}\text{H}_{10}\text{N}_3\text{OF}$: C, 64.19; H, 4.12; N, 17.28. Found C, 64.29; H, 4.31; N, 17.20.

N-phenyl-(4-cyano)-imino-picolinamide (4f): Light yellow coloured solid, Yield 76.3%, mp 172-176°C, R_f : 0.60 (hexane:chloroform, 9:1). IR (nujol) cm^{-1} : 2920 (C-H), 1626 (C = N), 1750 (C = O), 3358 (N-H), 1600, 1560, 1480 and 1460 (aromatic ring); $^1\text{HNMR}$ (400 MHz, CDCl_3 , ppm): δ 5.90 (s, 2H, -NCH₂), 3.68 (s, 1H, -NH), 6.51-6.70 (m, 4H, pyridine), 7.42-7.98 (m, 4H, phenyl protons); ESI-MS m/z : 249.1 (M^+), 250.9 ($M^+ + 2$); Anal. Calcd. for: $\text{C}_{14}\text{H}_{10}\text{N}_4\text{O}$: C, 67.20; H, 4.00; N, 22.40. Found C, 67.29; H, 4.08; N, 22.19.

N-phenyl-(4-bromo)-imino-picolinamide (4g): Dark yellow coloured solid, Yield 81.7%, mp 166-168°C, R_f : 0.52 (hexane:chloroform, 9:1). IR (nujol) cm^{-1} : 2914 (C-H), 1620 (C = N), 1736 (C = O), 3382 (N-H), 1600, 1590, 1520 and 1460 (aromatic ring); $^1\text{HNMR}$ (400 MHz, CDCl_3 , ppm): δ 5.78 (s, 2H, -NCH₂), 3.42 (s, 1H, -NH), 6.45-6.69 (m, 4H, pyridine), 7.48-7.88 (m, 5H, phenyl protons); ESI-MS m/z : 303.3 (M^+), 305.0 ($M^+ + 2$); Anal. Calcd. for: $\text{C}_{13}\text{H}_{10}\text{N}_3\text{OBr}$: C, 51.32; H, 3.29; N, 13.82. Found C, 51.36; H, 3.37; N, 13.91.

N-phenyl-(2-nitro)-imino-picolinamide (4h): Yellow coloured solid, Yield 84.0%, mp 182-187°C, R_f : 0.59

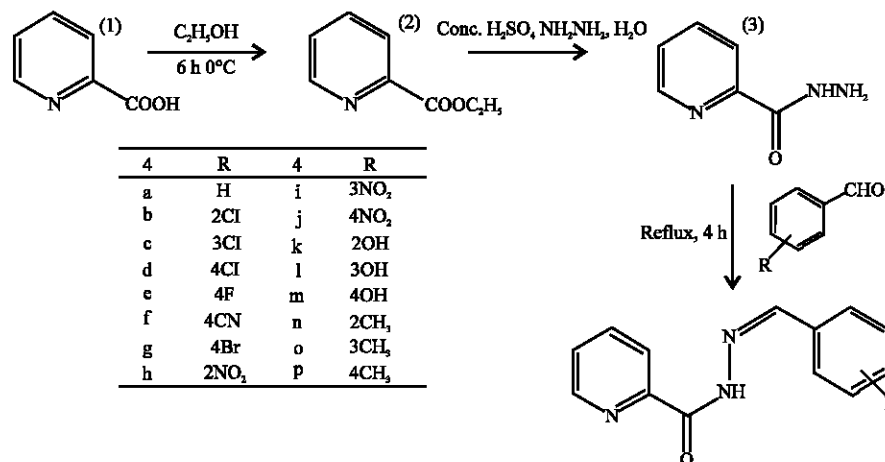


Fig. 1: Synthesis of substituted picolinic acid Schiff bases

Table 1: Screening of antifungal activity of substituted picolinamides

Compounds	ED ₅₀ (µg mL ⁻¹) at 3 df				
	<i>R. solani</i>	<i>A. alternata</i>	<i>S. rolfisii</i>	<i>F. oxysporum</i>	<i>M. phaseolina</i>
4a	117.3	115.5	127.8	141.5	149.7
4b	38.2	41.4	96.2	179.3	108.9
4c	29.1	33.9	91.7	163.7	109.5
4d	51.4	53.2	81.4	168.2	117.2
4e	87.2	70.7	72.8	183.3	100.5
4f	168.5	176.8	147.8	197.4	153.9
4g	113.8	191.6	116.6	178.2	123.5
4h	129.7	148.4	153.2	127.5	156.3
4i	136.3	213.2	135.8	128.9	180.3
4j	221.4	176.3	173.3	141.4	152.7
4k	74.4	67.6	56.3	161.6	144.8
4l	84.6	93.4	67.5	122.8	299.7
4m	99.4	81.7	72.7	159.7	149.3
4n	192.7	185.6	133.4	219.4	131.9
4o	180.3	188.4	130.7	202.2	158.2
4p	149.2	145.7	139.8	207.5	148.6
Bavistin*	>3.9	3.1	7.4	8.3	>8.2

*Bavistin as positive control

Table 2: Fungicidal activity of N-phenyl-(3-chloro)-imino-picolinamide (4c)

Fungi	ED ₅₀ (µg mL ⁻¹) at 3 df	χ ²	Regression equation	Fiducial limit (low-high)
4c				
<i>R. solani</i>	29.1±0.57	1.98	y = 1.28+2.65x	21.3-38.9
<i>A. alternata</i>	33.9±0.29	1.13	y = 1.91+3.34x	25.6-42.7
<i>S. rolfisii</i>	91.7±0.87	3.09	y = 1.12+2.18x	78.5-113.6
<i>F. oxysporum</i>	163.7±1.21	2.91	y = 0.88+2.60x	139.0-189.3
<i>M. phaseolina</i>	109.5±0.60	3.44	y = 1.54+2.50x	88.7-135.1

(hexane:chloroform, 8:2). IR (nujol) cm⁻¹: 2932 (C-H), 1628 (C=N), 1730 (C=O), 3370 (N-H), 1600, 1540, 1520 and 1480 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.68 (s, 2H, -NCH₂), 3.51 (s, 1H, -NH), 6.45-6.48 (m, 4H, pyridine), 7.72-8.12 (m, 4H, phenyl protons); ESI-MS m/z: 269.4 (M⁺), 271.1 (M⁺+2), 273.4 (M⁺+4); Anal. Calcd. for: C₁₃H₁₀N₄O₃; C, 57.78; H, 3.70; N, 20.74. Found C, 57.70; H, 3.71; N, 20.77.

N-phenyl-(3-nitro)-imino-picolinamide (4i): Yellow coloured solid, Yield 85.8%, mp 162-165°C, R_f: 0.63 (hexane:chloroform, 8:2). IR (nujol) cm⁻¹: 2920 (C-H), 1624

(C=N), 1744 (C=O), 3380 (N-H), 1600, 1590, 1500 and 1460 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.93 (s, 2H, -NCH₂), 3.56 (s, 1H, -NH), 6.48-6.90 (m, 4H, pyridine), 7.28-8.01 (m, 4H, phenyl protons); ESI-MS m/z: 269.1 (M⁺), 271.5 (M⁺+2); Anal. Calcd. for: C₁₃H₁₀N₄O₃; C, 57.78; H, 3.70; N, 20.74. Found C, 57.22; H, 3.51; N, 20.96.

N-phenyl-(4-nitro)-imino-picolinamide (4j): Yellow coloured solid, Yield 78.4%, mp 175-178°C, R_f: 0.58 (hexane:chloroform, 8:2). IR (nujol) cm⁻¹: 2922 (C-H), 1632

(C = N), 1740 (C = O), 3340 (N-H), 1560, 1500, 1490 and 1460 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.61 (s, 2H, -NCH₂), 3.72 (s, 1H, -NH), 6.59-6.72 (m, 4H, pyridine), 7.70-8.08 (m, 4H, phenyl protons); ESI-MS m/z: 269.2 (M⁺), 270.9 (M⁺+2), 273.0 (M⁺+4); Anal. Calcd. for: C₁₃H₁₀N₄O₃: C, 57.78; H, 3.70; N, 20.74. Found C, 57.43; H, 3.87; N, 20.57.

N-phenyl-(2-hydroxy)-imino-picolinamide (4k): Light yellow coloured solid, Yield 86.9%, mp 167-170°C, R_f: 0.49 (hexane:chloroform, 7:3). IR (nujol) cm⁻¹: 2940 (C-H), 1620 (C = N), 1756 (C = O), 3388 (N-H), 1595, 1582, 1570 and 1460 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.54 (s, 2H, -NCH₂), 13.12 (s, 1H, chelated-OH), 3.58 (s, 1H, -NH), 6.65-6.81 (m, 4H, pyridine), 7.77-8.15 (m, 4H, phenyl protons); ESI-MS m/z: 240.1 (M⁺), 263.0 (M⁺+Na); Anal. Calcd. for: C₁₃H₁₁N₃O₂: C, 64.73; H, 4.56; N, 17.42. Found C, 64.79; H, 4.42; N, 17.76.

N-phenyl-(3-hydroxy)-imino-picolinamide (4l): Light yellow coloured solid, Yield 72.6%, mp 172-175°C, R_f: 0.43 (hexane:chloroform, 7:3). IR (nujol) cm⁻¹: 2930 (C-H), 1625 (C = N), 1742 (C = O), 3380 (N-H), 1600, 1590, 1468 and 1455 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.44 (s, 2H, -NCH₂), 4.87 (s, 1H, -OH), 3.50 (s, 1H, -NH), 6.80-6.98 (m, 4H, pyridine), 7.68-8.10 (m, 4H, phenyl protons); ESI-MS m/z: 240.7 (M⁺), 242.1 (M⁺+2), 263.3 (M⁺+Na); Anal. Calcd. for: C₁₃H₁₁N₃O₂: C, 64.73; H, 4.56; N, 17.42. Found C, 64.61; H, 4.72; N, 17.81.

N-phenyl-(4-hydroxy)-imino-picolinamide (4m): Light yellow coloured solid, Yield 86.9%, mp 182-186°C, R_f: 0.38 (hexane:chloroform, 7:3). IR (nujol) cm⁻¹: 2936 (C-H), 1626 (C = N), 1740 (C = O), 3380 (N-H), 1600, 1590, 1500 and 1460 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.51 (s, 2H, -NCH₂), 4.85 (s, 1H, -OH), 3.56 (s, 1H, -NH), 6.86-6.90 (m, 4H, pyridine), 7.57-7.91 (m, 4H, phenyl protons); ESI-MS m/z: 240.0 (M⁺), 242.7 (M⁺+2), Anal. Calcd. for: C₁₃H₁₁N₃O₂: C, 64.73; H, 4.56; N, 17.42. Found C, 64.86; H, 4.99; N, 17.39.

N-phenyl-(2-methoxy)-imino-picolinamide (4n): Light yellow coloured solid, Yield 81.1%, mp 170-178°C, R_f: 0.47 (hexane: chloroform, 8:2). IR (nujol) cm⁻¹: 2975 (C-H), 1620 (C = N), 1702 (C = O), 3340 (N-H), 1560, 1536, 1512 and 1480 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.57 (s, 2H, -NCH₂), 3.87 (s, 1H, -OCH₃), 3.49 (s, 1H, -NH), 6.98-7.21 (m, 4H, pyridine), 7.84-8.23 (m, 4H, phenyl protons); ESI-MS m/z: 254.8 (M⁺), 256.2 (M⁺+2), Anal. Calcd. for: C₁₄H₁₃N₃O₂: C, 65.88; H, 5.10; N, 16.47. Found C, 65.81; H, 5.09; N, 16.39.

N-phenyl-(3-methoxy)-imino-picolinamide (4o): Light yellow coloured solid, Yield 74.7%, mp 195-198°C, R_f: 0.42 (hexane:chloroform, 8:2). IR (nujol) cm⁻¹: 2939 (C-H), 1634 (C = N), 1748 (C = O), 3310 (N-H), 1600, 1560, 1500 and 1490 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.64 (s, 2H, -NCH₂), 3.82 (s, 1H, -OCH₃), 3.52 (s, 1H, -NH), 6.82-6.98 (m, 4H, pyridine), 7.92-8.22 (m, 4H, phenyl protons); ESI-MS m/z: 254.0 (M⁺), 256.1 (M⁺+2), 277.4 (M⁺+Na), Anal. Calcd. for: C₁₄H₁₃N₃O₂: C, 65.88; H, 5.10; N, 16.47. Found C, 65.72; H, 5.15; N, 16.49.

N-phenyl-(4-methoxy)-imino-picolinamide (4p): Light yellow coloured solid, Yield 91.1%, mp 167-170°C, R_f: 0.50 (hexane:chloroform, 8:2). IR (nujol) cm⁻¹: 2979 (C-H), 1652 (C = N), 1700 (C = O), 3398 (N-H), 1562, 1540, 1508 and 1496 (aromatic ring); ¹HNMR (400 MHz, CDCl₃, ppm): δ 5.54 (s, 2H, -NCH₂), 3.89 (s, 1H, -OCH₃), 3.52 (s, 1H, -NH), 6.80-7.21 (m, 4H, pyridine), 7.47-7.90 (m, 4H, phenyl protons); ESI-MS m/z: 254.7 (M⁺), 277.1 (M⁺+Na), Anal. Calcd. for: C₁₄H₁₃N₃O₂: C, 65.88; H, 5.10; N, 16.47. Found C, 65.91; H, 5.01; N, 16.43.

Fungicidal evaluation: Synthesized picolinamide derivatives were evaluated for antifungal activity against five phytopathogenic fungi, *Rhizoctonia solani* ITCC 2775, *Alternaria alternata* ITCC 5501, *Sclerotium rolfsii* ITCC 5512, *Fusarium oxysporum* ITCC 1053 and *Macrophomina phaseolina* ITCC 3134 at various concentrations (Table 1). Preliminary screening of the synthesized compounds at higher concentration of 1000 µg mL⁻¹ revealed complete inhibition (100%) of fungal growth of all the test fungi. Therefore, the test concentration was further brought down to 200 µg mL⁻¹. As evidence from the data, at higher concentration of 200 µg mL⁻¹, N-phenyl-(3-chloro)-imino-picolinamide (4c) (89.4%) and N-phenyl-(2-chloro)-imino-picolinamide (4b) (89.0%) exhibited maximum fungal growth inhibition against *R. solani*. Chloro substituted picolinamide showed significantly high fungal growth inhibition against all the test fungi. N-phenyl-(3-fluoro)-imino-picolinamide possessed higher fungal growth inhibition against *A. alternata*. Nitro substituted phenylimino-picolinamide (4h) derivatives showed moderate fungal hyphal growth inhibition against *F. oxysporum* at the highest concentration (200 µg mL⁻¹). N-phenyl-(2-hydroxy)-imino-picolinamide (4k) exhibited significant fungal growth inhibition against *R. solani* (78.9%) followed by *A. alternata* (75.1%). Probit analysis of the fungal growth inhibition data revealed that all the synthesized imines possessed ED₅₀ below 299.7 µg mL⁻¹.

In terms of lethal dose, N-phenyl-(3-chloro)-imino-picolinamide exhibited maximum antifungal activity against *R. solani* (ED_{50} 29.1 $\mu\text{g mL}^{-1}$) and *A. alternata* (ED_{50} 33.9 $\mu\text{g mL}^{-1}$) (Table 2). 4-Chloro and 4-fluoro picolinamide exhibited ED_{50} in the range of 51.4-87.2 $\mu\text{g mL}^{-1}$ against the same fungi. N-phenyl-(2-hydroxy)-iminopicolinamide exhibited ED_{50} 56.3 $\mu\text{g mL}^{-1}$ against *S. rolfssii*.

DISCUSSION

Total sixteen picolinamide derivatives were synthesized both conventional and microwave assisted synthesis method. Under microwave irradiation technique the reaction completed within 3 min. Therefore, microwave assisted synthesis is preferable than conventional refluxing method. All the synthesized compounds were novel and their activities against soil borne plant pathogens had rarely been evaluated so far.

Chemical structures of the synthesized compounds (4a-p) were confirmed by IR, ^1H NMR, mass spectra and elemental analysis. For example, ^1H NMR spectrum of a representative compound (4a) exhibited sharp signal at δ 5.92 ppm belonging to $\text{N}=\text{CH}$ moiety, which is characteristic of any Schiff base. The aromatic protons of the compounds gave multiplet in the region δ 6.65-8.15 ppm. The IR spectrum indicated stretching vibration bands belonging to $\text{C}=\text{N}$ at 1620 cm^{-1} .

Additional stretching bands at 3380 and 1712 cm^{-1} directed the presence of $-\text{NH}$ and $-\text{CO}$ moiety. Structures of the compounds were further confirmed through their respective mass spectra. The molecular ion peak of the representative compound (4a) was observed at m/z 224.7 (M^+) and 226.2 ($\text{M}^+ + 2$) are fully supportive of the molecular formula $\text{C}_{13}\text{H}_{11}\text{N}_3\text{O}$. Molecular ion (m/z 224.7) further resulted fragment ion peaks at m/z 248.2 and 246.5 which originated as a result of sequential loss of aromatic (78 amu) and pyridine (79 amu) moiety from the parent ion

(Fig. 2). The structure of the synthesized compounds was further confirmed by their elemental analysis. Similarly, other novel substituted picolinamides were also characterized based on their physico spectral data. All the synthesized compounds were moderate to highly effective against *R. solani*. Chloro derivatives were most active as compared to nitro and hydroxy derivatives. Antifungal activity of all the test compounds was dose dependent and increased with increase in concentration. Incidentally, it is the first report of antifungal potentiality of picolinamide molecules. However, nicotinamides were reported to possess antimicrobial activities against human pathogens (Sharma *et al.*, 2009). More specifically, some other nicotinic acid amide bases were found to be active against *C. albicans* (Patel and Shaikh, 2010). The present study indicated that *R. solani* and *A. alternata* were most susceptible pathogen towards picolinamide molecules.

The activity was slightly increased following conversion of picolinic acid to picolinic acid ester but significantly increased after conversion of picolic acid ester to their Schiff bases. Among various derivatives, N-phenyl-(3-chloro)-imino-picolinamide (4c) and N-phenyl-(2-chloro)-imino-picolinamide (4b), with respective ED_{50} values of 29.1 and 38.2 $\mu\text{g mL}^{-1}$ were the most active. However, compared with the standard reference, Bavistin® (ED_{50} 3.90 $\mu\text{g mL}^{-1}$ against *R. solani*), the synthesized compounds were less active. A similar trend was evident with *A. alternata*. N-phenyl-(3-chloro)-imino-picolinamide (4c) and N-phenyl-(2-chloro)-imino-picolinamide (4b), with respective ED_{50} values of 29.1 and 38.2 $\mu\text{g mL}^{-1}$ were the most effective. N-phenyl-(2-hydroxy)-imino-picolinamide (4k) and N-phenyl-(4-hydroxy)-imino-picolinamide (4m), with respective hydroxy group at second and fourth position, were also active (ED_{50} 67.6-81.7 $\mu\text{g mL}^{-1}$).

The results of antifungal testing on the sixteen synthesized derivatives indicated that the substitution on aromatic ring showed a correlation with the antifungal

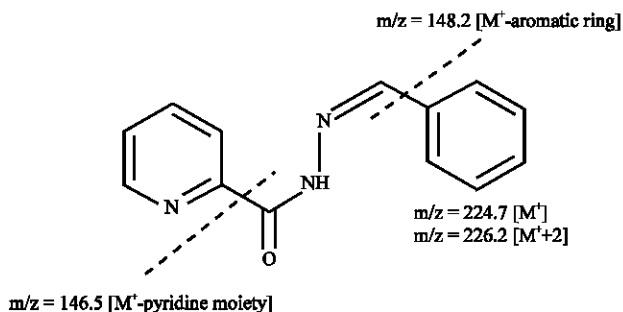


Fig. 2: Mass fragmentation pattern of compound 4a

activity. Structure-activity relationship revealed that methyl substitution at second, third, fourth position and un-substituted aromatic ring found to be least active against the test fungi. Best activity was observed for the halogen substituted compounds. Among the halogenated derivatives, chloro substituted compounds were most active. Moreover, substitution at third position of aromatic ring exhibited greater activity against *R. solani*. In case of other halogenated compounds, fluoro substituted derivative was moderately active against the same fungi. As for compounds 4h-4j with nitro substituted aromatic ring, it seemed that there were no obvious correlations between the position of the substituents on aromatic ring and antifungal activity. However, hydroxy derivatives were most active against *S. rolfssii*. Hydroxy substitution at second position of phenyl ring exhibited higher activity followed by substitution at third and fourth position. Therefore, the antifungal activity of the synthesized picolinamide molecules was selective and activity depended on specific positional substitution of aromatic ring.

The present study has demonstrated the potential effect of picolonamide derivatives as fungicide against *R. solani*. Standard fungicidal formulation may be developed based on the most active chloro substituted derivative as active ingredient. Structure activity relationship may be useful for further designing of potential novel molecules with more substituents on aromatic ring.

ACKNOWLEDGMENTS

The authors thank Head, Division of Agricultural Chemicals, Indian Agricultural Research Institute, New Delhi, for providing financial assistance.

REFERENCES

- Aggarwal, N., R. Kumar, P. Dureja and D.S. Rawat, 2009. Schiff bases as potential fungicides and nitrification inhibitors. *J. Agric. Food Chem.*, 57: 8520-8525.
- Borawska, M.H., S.K. Czechowska, R. Markiewicz, J. Paćka, R. Swislocka and W. Lewandowski, 2008. Antimicrobial activity and cytotoxicity of picolinic acid and selected picolinate as new potential food preservatives. *Pol. J. Food Nutr. Sci.*, 58: 415-418.
- Cai, S., K. Sato, T. Shimizu, S. Yamabe, M. Hiraki, C. Sano and H. Tomioka, 2006. Antimicrobial activity of picolinic acid against extracellular and intracellular *Mycobacterium avium* complex and its combined activity with clarithromycin, rifampicin and fluoroquinolones. *J. Antimicrob. Chemother.*, 57: 85-93.
- Coggan, S.E., G.A. Smythe, A. Bilgin and R.S. Grant, 2009. Age and circadian influences on picolinic acid concentrations in human cerebrospinal fluid. *J. Neurochem.*, 108: 1220-1225.
- Dangi, R.R., N. Hussain, A. Joshi, G. Pemawat and G.L. Talesara, 2011. Design, facile synthesis and biological evaluation of quinazoline containing pyrazolothiazoly, triazinone and their ethoxyphthalimide derivatives. *Indian J. Chem.*, 50: 1165-1172.
- Dazzi, C., G. Candiano, S. Massazza, A. Ponzetto and L. Varesio, 2001. New high-performance liquid chromatographic method for the detection of picolinic acid in biological fluids. *J. Chromatogr. B: Biomed. Sci. Appl.*, 751: 61-68.
- Dhar, D.N. and C.L. Taploo, 1982. Schiff bases and their applications. *J. Sci. Ind. Res.*, 41: 501-506.
- Dowd, P.F., 1999. Relative inhibition of insect phenoloxidase by cyclic fungal metabolites from insect and plant pathogens. *Nat. Toxins*, 7: 337-341.
- Hamaker, J.W., H. Johnston, R.T. Martin and C.T. Redemann, 1963. A picolinic acid derivative: A plant growth regulator. *Science*, 141: 363-363.
- Hoveyda, H.R., V. Karunaratne, C.J. Nichols, S.J. Rettig, A.W. Stephens and C. Orvig, 1998. Tripodal trisamides based on nicotinic and picolinic acid derivatives. *Can. J. Chem.*, 76: 414-425.
- Jarrahpour, A., D. Khalili, E. De Clercq, C. Salmi and J.M. Brunel, 2007. Synthesis, antibacterial, antifungal and antiviral activity evaluation of some new bis-Schiff bases of isatin and their derivatives. *Molecules*, 12: 1720-1730.
- Jesmin, M., M.M. Ali and J.A. Khanam, 2010. Antitumour activities of some schiff bases derived from benzoin, salicylaldehyde, amino phenol and 2,4 dinitrophenyl hydrazine. *Thai J. Pharm. Sci.*, 34: 20-31.
- Koczon, P., J. Piekut, M. Borawska, R. Swislocka and W. Lewandowski, 2005. The relationship between chemical structure and antimicrobial activity of selected nicotinate, *p*-iodobenzoates, picolinate and isonicotinate. *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, 61: 1917-1922.
- Lingala, S., R. Nerella and K.R.S.S. Rao, 2011. Synthesis, antimicrobial and anthelmintic activity of some novel benzimidazole derivatives. *Pharm. Chem.*, 3: 344-352.
- Musk Jr., D.J. and P.J. Hergenrother, 2008. Chelated iron sources are inhibitors of *Pseudomonas aeruginosa* biofilms and distribute efficiently in an *In vitro* model of drug delivery to the human lung. *J. Applied Microbiol.*, 105: 380-388.
- Newell, K.W., A.D. Ross and R.M. Renner, 1984. Phenoxy and picolinic acid herbicides and small-intestinal adenocarcinoma in sheep. *Lancet*, 324: 1301-1305.

- Patel, N.B. and F.M. Shaikh, 2010. New 4-thiazolidinones of nicotinic acid with 2-amino-6-methylbenzothiazole and their biological activity. *Sci. Pharma.*, 78: 753-765.
- Sharma, M.C., N.K. Sahu, D.V. Kohli, S.C. Chaturvedi and S. Sharma, 2009. Synthesis, characterization and biological activities of some 1-(Nicotinylamino)-2-substituted azetidione-4-ones as potential antibacterial agents. *Dig. J. Nanomater. Biostruct.*, 4: 361-367.
- Tang, Y.Z. and Z.Q. Liu, 2007. The antioxidant effect of hydroxyl-substituent Schiff bases on the free-radical-induced hemolysis of human erythrocytes. *Cell Biochem. Funct.*, 25: 149-158.
- Upreti, G.C., R.P. Singh, J. Verma, P.L. Bhatia and K.G. Gollakota, 1969. The effects of some alpha picolinic acid derivatives on growth and sporulation of bacilli. *Biochem. Biophys. Res. Commun.*, 35: 611-618.
- Yang, H.J., W.H. Sun, Z.L. Li and M. Zhi, 2002. The rapid synthesis of Schiff-base without solvent under microwave irradiation. *Chinese Chem. Lett.*, 13: 3-6.