DOI:10.1021/jf904144e

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Synthesis of Nalidixic Acid Based Hydrazones as Novel Pesticides

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Thirty-one substituted hydrazones of nalidixic acid hydrazide were synthesized and characterized by spectral techniques. These compounds were evaluated for various biological activities, namely, fungicidal, insecticidal, and nitrification inhibitory activities. The antifungal activity was evaluated against five pathogenic fungi, namely, *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Alternaria porii*. They showed maximum inihibition against *A. porii* with ED_{50} = 34.2−151.3 μg/mL. The activity was comparable to that of a commercial fungicide, hexaconazole (ED_{50} = 25.4 μg/mL). They were also screened for insecticidal activity against third-instar larvae of *Spodoptera litura* and adults of *Callosobruchus maculatus* and *Tribollium castaneum*. Most of them showed 70−100% mortality against *S. litura* through feeding method at 0.1% dose. These compounds were not found to be effective nitrification inhibitors.

KEYWORDS: Nalidixic acid; hydrazones; antifungal activity; insecticidal activity; nitrification inhibitory activity

INTRODUCTION

The major constraint limiting higher agricultural productivity is the onslaught of insect pests and diseases. Over 30% yield losses in major crops are caused by pathogenic fungi and insect pests (*1*). These soil-inhabiting fungi cause wilt diseases, leaf wilting, dry root rot, yellowing, and eventually plant death (*2*). Among these *Fusarium oxysporum* and *Alternaria porii* are the most devastating fungi, especially for banana plant and onion crop (*3*, *4*). Insect pests affect the growth of plants and reduce the photosynthetic ability of crop. On most crops, damage arises from extensive feeding by larvae, which leads to complete stripping of the plants (*5*). Among all insect pests, *Spodoptera litura* is an extremely serious polyphagous pest of several cultivated crops, the larvae of which can defoliate many economically important crops (*6*). This pest has developed resistance to many conventional and currently available insecticides (*7*). Hence, there is a need to search for an alternate pesticide that can fit into farmers' budgets as well as an integrated pest management (IPM) program.

Another major problem of global concern is the low yields of crops due to low efficiency of fertilizers inputs, which results in U.S. $16 billion annual loss of nitrogen (N) fertilizers worldwide (*8*). 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 (*9*, *10*). Regulation of urea hydrolysis and nitrification in agricultural systems has been a major strategy in overcoming these losses. The use of nitrification inhibitors minimizes these effects. Nitrapyrin, dicyandiamide, etridiazole, etc., are the common commercial nitrification inhibitors. The high costs of development and subsequent registration of effective inhibitors are serious issues in their extensive use (*11*), underscoring a need to develop simple, efficient, economical, and safe nitrification inhibitors.

Nalidixic acid (1,8-naphthapyridine derivative) is the first synthetic quinolone antibiotic introduced in the 1960s. Structure−activity relationships (SAR) of compounds based on nalidixic acid have led to a large group of synthetic bioactive molecules known as the quinolones (*12*). Hydrazide−hydrazones have been claimed to exhibit appreciable antimicrobial activity (*13*−*15*). Furthermore, a number of hydrazide−hydrazone derivatives have been claimed to possess interesting antibacterial, antifungal (*16*, *17*), anticonvulsant (*18*), anti-inflammatory (*19*), antimalarial (*20*), and antituberculosis activities (*21*). The hydrazone derivatives are used as active ingredients in the method for controlling agricultural and horticultural insect pests (*22*). Therefore, to explore the biopotential of nalidixic acid derivatives, the present study was undertaken to synthesize a series of novel nalidixic acid based hydrazones and to evaluate them as fungicides, insecticides, and nitrification inhibitors.

MATERIALS AND METHODS

Chemicals and Instruments. All of 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 $60F_{254}$, and the spots were visualized either under UV or by iodine vapor. Melting points were determined on a JSW melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin-Elmer

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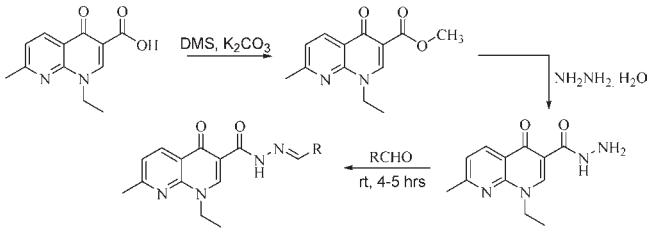
 Published on Web 02/04/2010

model 2000 FT-IR spectrophotometer as KBr pellet, and values are expressed as ν_{max} cm^{-1}. Mass spectra were recorded on a JEOL (Japan) JMS-DX303 and micromass LCT, mass spectrometer/data system. The



1. R= 2-F-C_6H_4
2. R= 4-F-C_6H_4
3. R= 3-Br-C_6H_4
4. R= 2-Cl-C_6H_4
5. R= 3-Cl-C_6H_4
6. R= 4-Cl-C_6H_4
7. R= 2-NO_2-C_6H_4
8. R= 3-NO_2-C_6H_4
9. R= 4-NO_2-C_6H_4
10. R= 4-OH-C_6H_4
11. R= 3-OCH_3-C_6H_4
12. R= 4-OCH_3-C_6H_4
13. R= 2-CH_3-C_6H_4
14. R= 3-CH_3-C_6H_4
15. R= 4-CH_3-C_6H_4
16. R= 4-$CH(CH_3)_2$-C_6H_4
17. R= 2,4-$(Cl)_2$-C_6H_3
18. R= 2,6-$(Cl)_2$-C_6H_3
19. R= 2,4-$(OH)_2$-C_6H_3
20. R= 2,4-$(OCH_3)_2$-C_6H_3
21. R= 3,4-$(OCH_3)_2$-C_6H_3
22. R= 3-(OCH_3)-4-(OH)-C_6H_3
23. R= 3-(OC_2H_5)-4-(OH)-C_6H_3
24. R= 3,4,5-$(OCH_3)_3$-C_6H_2
25. R= 2-pyridyl
26. R= 1-napthyl
27. R= 2-napthyl
28. R= 9-anthryl
29. R= cyclohexyl
30. R= admantyl
31. R= crotonyl

Figure 1. Synthesis of substituted nalidixic acid based hydrazones (**1**−**31**).

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Spectrospin spectrometer (400 and 75.5 MHz), using tetramethylsilane as an internal standard. The chemical shift values were recorded on 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 Hydrazones* (***1*−*31***). Nalidixic acid hydrazide was prepared according to a reported procedure (*12*). To a stirred solution of nalidixic acid hydrazide (5 mmol) in methanol (10 mL) were added substituted benzaldehydes (5.5 mmol), and the mixture was stirred at room temperature for 3 h. The precipitate thus obtained was filtered, washed with a minimum amount of cold methanol, and recrystallized from ethanol (**Figure 1**).

1-Ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-fluoro-benzylidene)-hydrazide (***1***): yield, 85%; white solid; melting point, 280 °C; IR, 3449, 2989, 1676, 1608, 1568, 1231, 756; ^1H NMR δ 1.53 (t, 3H, N−$CH_2\underline{CH_3}$), 2.71 (s, 3H, 7-CH_3), 4.60 (q, 2H, N−$\underline{CH_2}$), 7.10−7.17 (m, 1H, H-3-phenyl), 7.19−7.27 (m, 1H, H-5-phenyl), 7.33−7.35 (m, 1H, H-4-phenyl), 7.37 (d, J = 8.40 Hz, 1H, H-6-naphthyridine), 7.49−7.56 (m, 1H, H-6-phenyl), 8.56 (s, 1H, H-2-naphthyridine), 8.67 (d, J = 5.20 Hz, 1H, H-5-naphthyridine), 9.03 (s, 1H, N═CH), 13.23 (s, 1H, NH); HRMS calculated for $C_{19}H_{17}FN_4O_2$, 352.1336, found, 353.1308 ($M^+ + H$).

In Vitro Antifungal Activity. The antifungal activity of the compounds was evaluated in vitro using a food poison technique (*23*).

In Vitro Insecticidal Activity. The biological assay was conducted against third-instar larvae of *Spodoptera litura* (7 ± 1 day old) and adults of *Callosobruchcus maculatus* (1−2 days old) and *Tribollium castaneum* (12 ± 1 days old). Adults of *C. maculatus* and *T. castaneum* were obtained

Table 1. Antifungal Activity Data of Nalidixic Acid Based Hydrazones (**1**−**31**) against Five Pathogenic Fungi[a]

		antifungal activity									
		RB		SR		RS		FO		AP	
compd	R	α	β	α	β	α	β	α	β	α	β
1	2-fluorophenyl	668.0	2.5	539.7	1.6	358.7	2.9	107.4	3.3	78.3	2.4
2	4-fluorophenyl	243.7	4.7	219.9	5.2	234.5	2.7	79.9	0.7	64.8	4.8
3	2-chlorophenyl	593.2	3.0	570.4	3.3	245.6	4.0	143.3	5.6	90.1	3.8
4	3-chlorophenyl	503.9	1.8	438.8	2.4	393.9	4.0	131.9	4.5	98.8	2.1
5	4-chlorophenyl	370.6	1.1	437.7	1.0	307.5	3.4	118.7	1.2	87.2	2.4
6	3-bromophenyl	627.6	4.0	380.2	5.6	438.8	1.4	209.2	3.3	125.3	3.7
7	2-nitrophenyl	610.5	4.2	654.6	3.7	328.6	4.2	168.8	5.0	71.3	1.5
8	3-nitrophenyl	271.5	1.0	286.9	5.6	244.7	1.9	212.2	2.9	89.0	1.4
9	4-nitrophenyl	575.3	0.7	463.6	0.5	225.7	0.2	125.3	3.7	70.1	5.3
10	4-hydroxyphenyl	307.8	2.9	364.0	5.6	330.4	1.2	181.2	4.9	145.9	3.5
11	3-methoxyphenyl	209.7	2.3	242.3	4.9	157.4	3.8	63.1	0.0	57.6	0.2
12	4-methoxyphenyl	300.7	4.3	320.2	5.0	238.2	1.1	151.5	5.2	121.1	3.4
13	2-methylphenyl	533.4	3.4	346.0	3.9	408.3	1.8	128.6	5.8	109.2	5.3
14	3-methylphenyl	322.1	1.7	297.7	1.7	301.2	4.2	103.1	5.1	66.9	1.0
15	4-methylphenyl	378.0	5.3	374.4	4.3	372.1	1.2	195.1	1.8	87.3	2.5
16	4-isopropylphenyl	453.3	1.6	463.6	0.5	252.5	2.4	277.1	3.0	124.5	1.7
17	2,4-dichlorophenyl	494.6	4.3	540.6	4.0	353.6	1.2	121.1	2.5	119.4	1.8
18	2,6-dichlorophenyl	255.1	2.8	282.5	3.1	297.7	4.1	114.2	3.4	87.2	1.0
19	2,4-dihydroxyphenyl	649.9	4.5	396.8	2.3	341.6	2.9	196.5	4.6	143.8	5.3
20	2,4-dimethoxyphenyl	382.2	2.9	374.8	1.6	188.2	4.3	176.3	3.1	131.1	3.0
21	3,4-dimethoxyphenyl	284.7	3.7	358.7	5.6	171.7	4.4	136.6	3.4	102.1	3.4
22	4-hydroxy-3-methoxyphenyl	528.0	2.4	459.7	1.1	316.7	3.4	376.8	1.6	128.2	3.2
23	4-hydroxy-3-ethoxyphenyl	399.9	1.0	384.3	1.4	356.2	2.1	184.8	4.9	92.1	5.3
24	3,4,5-trimethoxyphenyl	201.0	4.6	186.0	2.7	173.6	4.7	102.5	2.6	79.4	2.3
25	2-pyridyl	371.9	3.7	197.7	2.5	170.4	1.7	79.1	5.3	77.3	0.9
26	1-naphthyl	315.7	2.8	259.0	2.7	226.5	1.7	134.5	2.7	115.3	3.7
27	2-naphthyl	256.2	4.5	202.4	5.2	191.6	4.9	89.5	2.6	75.6	3.2
28	9-anthryl	389.7	1.4	428.6	2.9	374.3	2.0	222.1	0.9	151.3	1.9
29	cyclohexyl	188.2	4.3	177.1	1.2	166.5	4.5	62.6	0.3	34.2	2.1
30	adamantyl	638.2	2.4	523.9	5.0	405.4	3.8	258.5	3.6	132.7	2.8
31	crotonyl	324.0	4.7	203.8	1.3	247.5	2.6	95.4	5.3	89.8	5.9
hexaconazole		4.4	1.2	13.0	1.0	18.3	2.1	20.6	1.6	25.5	1.2

[a] RB, *Rhizoctonia bataticola*; SR, *Sclerotium rolfsii*; RS, *Rhizoctonia solani*; FO, *Fusarium oxysporum*; AP, *Alternaria porii*; α, ED_{50} value (μg/mL), calculated from mean percentage inhibition, which is an average of four replicates and its standard deviation (±) ranging from ±0.22 to ±5.87; β, chi square for heterogeneity (tabular value at 0.05 level) = 5.99 (degrees of freedom = 3).

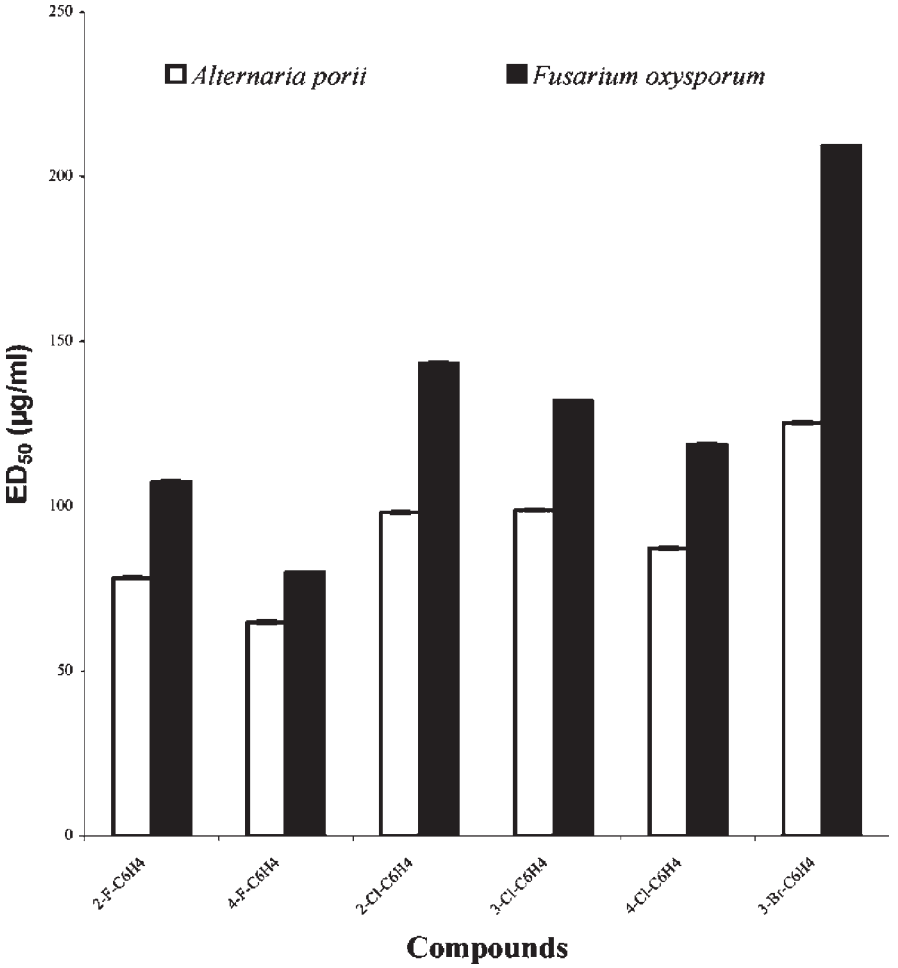


Figure 2. Antifungal activity of halogen-substituted derivatives (**1**−**6**) against *A. porii* and *F. oxysporum* (error bars shows standard deviation).

from the Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi. India. Insects were reared on green gram (*Vigna radiate*) and wheat at 27 ± 1 °C and 70% relative humidity. Insecticidal activity of the synthesized compounds was evaluated by film residue method and residue method for contact and feeding toxicity, respectively. The larvae of *S. litura* were collected from castor fields of IARI and were reared in the laboratory at 27 ± 1 °C and 70% relative humidity according to a reported method (*24*).

Bioassays. *Film Residue Method.* All synthesized (**1**−**31**) hydrazones were weighed and dissolved in dichloromethane to prepare 0.1% stock solution. One milliliter of a 0.1% solution of various compounds was added to each Petri plate (90 mm). Petri plates with test solution were rotated vigorously to prepare uniform film and were allowed to dry for 3−5 min. The experiments were performed in triplicates along with solvent as a control and reference insecticide as malathion. Ten adults (2−5 days old) were released in each Petri plate and were kept at 27 ± 1 °C with 70% relative humidity. Mortality was observed after 24 h. Adults were considered to be dead if they failed to respond to stimulus by touch.

Residue Method. Grains of green gram (*V. radiate*) were treated with test compounds dissolved in dichloromethane (0.6 mg in 5 mL) at a dose of 20 mg of active ingredient kg^{-1} of grains (0.002% of active ingredient). The treated grains (10 g) were kept in a plastic box, and 10 adults of *T. castaneum* were released in it. The experiment was conducted in triplicate with solvent as control. Mortality was observed after 48 h.

Feeding Method. The castor leaf was dipped in a 0.1% solution of synthesized compounds (**1**−**31**) for 2 s and then air-dried. Moist filter paper was placed in glass Petri plates (9 cm diameter) on which treated leaf disks were kept. Larvae of *S. litura* prestarved for 4 h were released individually into each Petri plates. Thirty replications were kept for each treatment. Solvent was used as control. Mortality was observed after 24 h.

Topical Treatment. The 0.1% stock solution of various compounds was prepared in dichloromethane. Two microliters of each compound was applied on the ventral side of the *S. litura* larvae. Ten treated larvae were released in glass bottles, and fresh tender castor leaves were given as food. Each treatment was kept in triplicate, and solvent was used as control. Mortality was observed after 24 h.

Nitrification Inhibitory Activity. The above synthesized compounds were also evaluated for their effect on nitrification inhibition at a 5% dose according to the following reported method.

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

Experiment. The experiments were conducted following a completely randomized design (CRD) with three replicates. The test chemicals and reference inhibitor were tested at a 5% dose of applied urea-N along with a urea alone control. The samples were incubated in 100 mL capacity plastic beakers (50 g of air-dried soil was taken per beaker). A 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 of the treatments including control, the same volume of acetone was added. After thorough mixing, 10 mg of urea-N (200 mg of urea-N kg^{-1} of soil) in aqueous solution was added and mixed thoroughly. Distilled water was added to each beaker to maintain the moisture at 50% water-holding capacity of the soil. The controls were similarly processed with urea alone at 200 mg kg^{-1} urea-N level without adding any test/reference inhibitor. All of the beakers were accurately weighed, labeled, and kept at 28 ± 1 °C with 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 (*25*).

Sampling and Estimation of Ammonium-N, Nitrite-N, and Nitrate-N from the Soil Samples. The soil samples (5 g) were withdrawn on the 21st day of incubation and extracted with aqueous sodium sulfate (50 mL, 1 M) by shaking on a reciprocal shaker for 1 h. The soil samples were filtered and estimated for ammonium-N, nitrite-N, and nitrate-N. 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 (*26*, *27*), respectively. The contents of ammonium-N, nitrite-N, and nitrate-N were obtained from the standard curves and expressed in milligrams per kilogram. The nitrification rate (NR) and percent nitrification inhibition (NI) were calculated using Sahrawat's formulas (*26*).

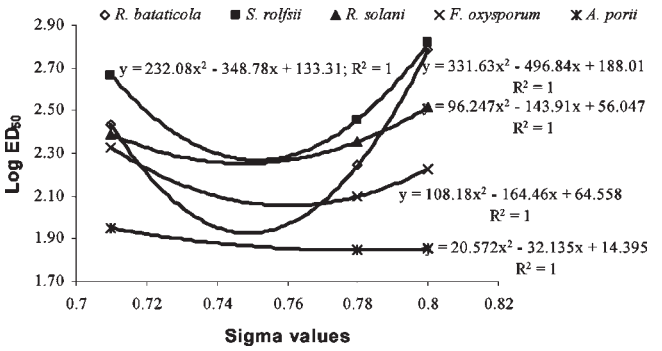


Figure 3. Plot of log ED_{50} values for all test fungi versus electronic parameters (σ_o, σ_m, σ_p) for *o*-, *m*-, *p*-nitrophenyl-substituted nalidixic acid hydrazones (**7**−**9**).

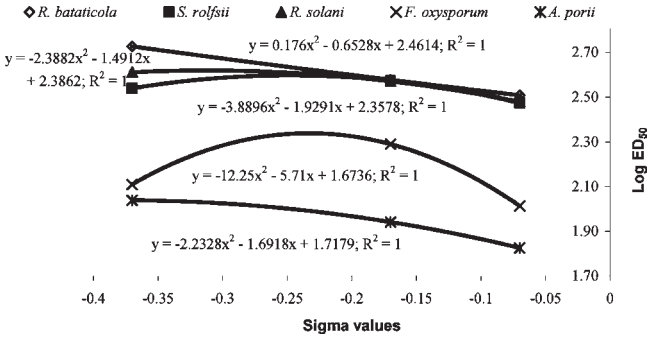


Figure 4. Plot of log ED_{50} value for all test fungi versus electronic parameters (σ_o, σ_m, σ_p) for *o*-, *m*-, *p*-methylphenyl-substituted nalidixic acid hydrazones (**13**−**15**).

RESULTS AND DISCUSSION

Methyl ester of naldixic acid was synthesized (*28*), which was further hydrazinolyxed to naldixic acid hydrazide (*12*). The hydrazide was condensed with various aldehydes to afford 31 substituted hydrazones (**Figure 1**). All of the hydrazones were characterized using spectral techniques and microanalysis, and the respective data are provided as Supporting Information. The spectral and analytical data for the four known hydrazones were in agreement with that reported in the literature (*29*).

In Vitro Antifungal Activity. All of the synthesized hydrazones (**1**−**31**) were screened for fungicidal activity against *Rhizoctonia bataticola*, *Sclerotium rolfsii*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Alternaria porii* by the poisoned food technique, and their calculated ED_{50} values are reported in **Table 1**. Most of the compounds showed maximum inhibition against *A. porii* (ED_{50} = 34.2−151.3 μg/mL) followed by *F. oxysporum* (ED_{50} = 62.6−376.8 μg/mL). Compound **29** (ED_{50} = 34.2−188.2 μg/mL) showed maximum antifungal activity against all test fungi, being effective against *A. porii* (ED_{50} = 34.2 μg/mL) comparable with hexaconazole (ED_{50} = 25.5 μg/mL), a commercial fungicide. Fluoro-substituted analogues were more effective than the chloro and bromo substituents irrespective of their positions (**Figure 2**). Sigma values for *o*-, *m*-, and *p*-nitrophenyl/methylphenyl-substituted nalidixic acid hydrazones were plotted against corresponding log ED_{50} values (**Figures 3** and **4**). The sigma values were low to moderate for *o*-NO_2 (σ_o = 0.8), *m*-NO_2 (σ_m = 0.71), and *p*-NO_2 (σ_p = 0.78) and for *o*-CH_3 (σ_o = −0.37), *m*-CH_3 (σ_m = −0.07), and *p*-CH_3 (σ_p = −0.17) (*30*). In all cases a quadratic relationship with a very high R^2 (coefficient of determination) value was observed. A perusal of the trend equations revealed the significantly high antifungal activity of hydrazone derivatives with an electron-withdrawing nitro group

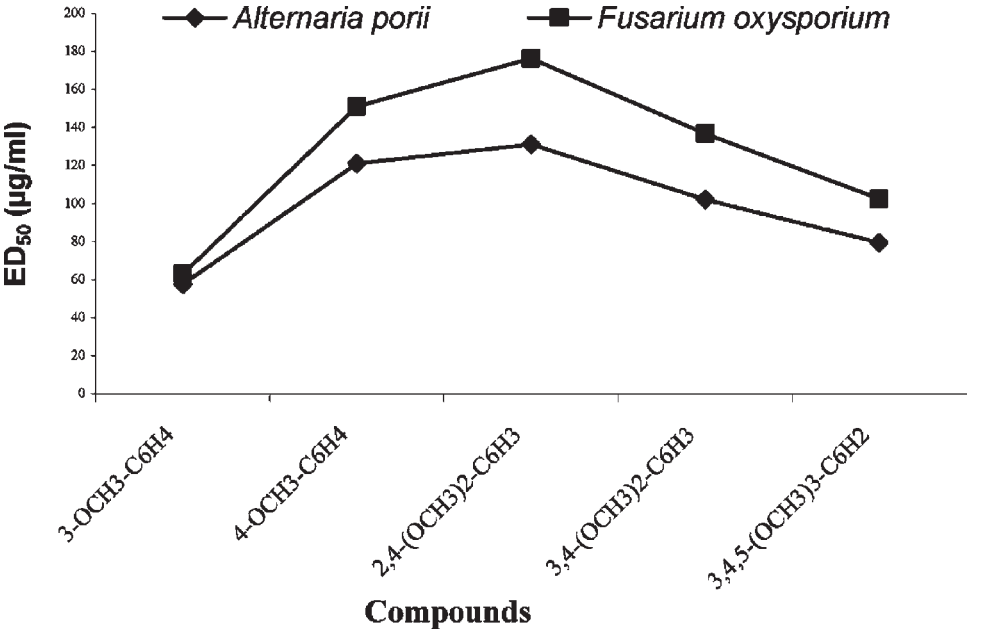


Figure 5. Antifungal activity profile of aryl derivatives (**11**, **12**, **20**, **21**, **24**) with varied number and position of methoxy group(s) against *A. porii* and *F. oxysporum*.

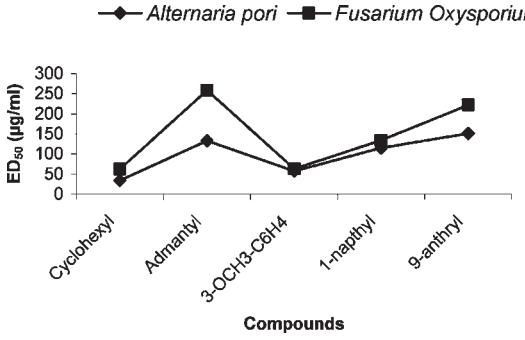


Figure 6. Effect of increase in size of ring on antifungal activity.

Table 2. Percent Mortality of Nalidixic Acid Based Hydrazones (**1**−**31**) against *Spodoptera litura*, *Callosobruchus maculatus*, and *Tribollium castaneum*

		mortality (%)				
		S. litura		*T. castaneum*		*C. maculatus*
compd	R	feeding	contact	feeding	contact	contact
1	2-fluorophenyl	90	10	10		30
2	4-fluorophenyl	50				10
3	2-chlorophenyl	10	10	10		30
4	3-chlorophenyl	90				10
5	4-chlorophenyl	40				10
6	3-bromophenyl	20	10			20
7	2-nitrophenyl	100	30	20		60
8	3-nitrophenyl	80				10
9	4-nitrophenyl	90	10			10
10	4-hydroxyphenyl	70	10			40
11	3-methoxyphenyl	10				10
12	4-methoxyphenyl	70		30		70
13	2-methylphenyl	0				10
14	3-methylphenyl	80				60
15	4-methylphenyl	70	10			20
16	4-isopropylphenyl	10				20
17	2,4-dichlorophenyl	100				40
18	2,6-dichlorophenyl	100	10			30
19	2,4-dihydroxyphenyl	40				25
20	2,4-dimethoxyphenyl	100	10			12
21	3,4-dimethoxyphenyl	40				10
22	4-hydroxy-3-methoxyphenyl	100	20			20
23	4-hydroxy-3-ethoxyphenyl	90	10			22
24	3,4,5-trimethoxyphenyl	90	10			10
25	2-pyridyl	100				10
26	1-naphthyl	90	10			0
27	2-naphthyl	70	10	10		0
28	9-anthryl	20	10	15		10
29	cyclohexyl	70		15		0
30	adamantyl	50	10	10		0
31	crotonyl	60				0
cypermethrin		100				
malathion				100	100	100

at the para position and an electron-releasing methyl group at the meta position. The above observation was further supported by the activity data of the compounds having methoxy group(s) at meta and/or para positions (**Figure 5**). An increase in activity was observed when a hydroxyl group (compound **10**) was replaced with a methoxy group (compound **12**) in the aromatic ring. Increase in size of either saturated ring (compound **29**, having a 6-membered ring, and compound **30**, having a 10-membered ring) or unsaturated ring (compound **26**, having a 10-membered ring, and compound **28**, having a 14-membered ring) resulted in a decrease in fungicidal activity (**Figure 6**). The compounds with unsaturated aliphatic and heterocyclic substitution showed higher fungicidal activity (compounds **25** and **31**, ED_{50} = 77.2−95.4 μg/mL) against *F. oxysporum* and *A. porii*. Compounds with a naphthyl substituent (compounds **26** and **27**) at the β-position (ED_{50} = 75.5−256.2 μg/mL) were found to be more active as compared to substitution at the α-position (ED_{50} = 115.2−315.7 μg/mL).

Table 3. Effect of Nalidixic Acid Based Hydrazones (**1**−**31**) on Ammonium-N, Nitrite-N, and Nitrate-N Contents and Rate of Nitrification

compd	R	ammonia-N (mg/kg)	nitrite-N (mg/kg)	nitrate-N (mg/kg)	nitrification rate (%)
1	2-fluorophenyl	24		172	95
2	4-fluorophenyl	16		144	95
3	2-chlorophenyl	25		127	92
4	3-chlorophenyl	15		147	91
5	4-chlorophenyl	55		100	81
6	3-bromophenyl	30		117	80
7	2-nitrophenyl	9		164	98
8	3-nitrophenyl	14		126	96
9	4-nitrophenyl	17		181	97
10	4-hydroxyphenyl	8		165	96
11	3-methoxyphenyl	13		170	93
12	4-methoxyphenyl	20		179	96
13	2-methylphenyl	11		165	94
14	3-methylphenyl	22		138	86
15	4-methylphenyl	13		185	97
16	4-isopropylphenyl	21		151	94
17	2,4-dichlorophenyl	20		125	86
18	2,6-dichlorophenyl	19		155	94
19	2,4-dihydroxyphenyl	10		154	94
20	2,4-dimethoxyphenyl	15		144	90
21	3,4-dimethoxyphenyl	17		149	95
22	4-hydroxy-3-methoxyphenyl	22		133	93
23	4-hydroxy-3-ethoxyphenyl	19		112	93
24	3,4,5-trimethoxyphenyl	14		142	96
25	2-pyridyl	11		159	93
26	1-naphthyl	26		133	84
27	2-naphthyl	2		195	99
28	9-anthryl	8		174	96
29	cyclohexyl	9		175	95
31	crotonyl	13		151	92
urea		11	2	184	94

In Vitro Insecticidal Activity. All of these compounds were also evaluated for in vitro insecticidal activity against the lepidopteran insect pest of field crops and the coleopteran insect pest of stored grains at 0.1% dose through contact as well as feeding method. Bioassay of the compounds was conducted against third-instar larvae of *S. litura* and adults of storage insects *C. maculatus* and *T. castaneum*. Most of the compounds showed insecticidal activity (mortality 80−100%) against *S. litura* when tested through the feeding method. The insecticidal activity of these compounds was comparable to that of cypermethrin, a commercial pyrethroid insecticide when tested at a 0.1% dose. No mortality was observed when these compounds were tested by topical treatment (i.e., through direct penetration on the insects) against *S. litura* Compounds tested against adults of *C. maculatus* by contact method showed adult mortality ranging from 20 to 70%. None of the compounds showed mortality against *T. castaneum* when tested either by contact or by feeding method. The results of bioassay (**Table 2**) indicated that of the various tested compounds, compound **7** showed the best toxicity against *S. litura* as well as *C. maculatus* at 0.1% dosage. This compound showed complete mortality against *S. litura* and 60 and 20% mortality against *C. maculatus* and *T. castaneum*, respectively. Apart from compound

7, compounds **17**, **18**, **20**, **22**, and **25** were also found to be very effective against *S. litura* as they also showed 100% mortality. They showed moderate to good mortality against *C. maculatus* but did not show any mortality against *T. castaneum*. Compounds **3**, **11**, **13**, and **16** showed least toxicity against all of the insect pests. In general, it was found that all of the compounds were effective against *S. litura* larvae, the voracious feeding stage, when mixed in the food, whereas these compounds were not effective against hard-body storage insects such as *C. maculatus* and *T. castaneum* through either contact or feeding method.

Nitrification Inhibitory Activity. Results obtained in the in vitro soil incubation study are reported in **Table 3**. All of these compounds are not significantly active as nitrification inhibitors. Ammonium-N content was in the range of 10−55 mg/kg. Compound **5** showed maximum ammonium-N content (55 mg/kg) as compared to urea (11 mg/kg). The nitrite-N content was below 0.5 mg/kg in all of the treatments, whereas urea showed 2 mg/kg. Nitrate-N content for the test chemicals varied from 100 to 195 mg/kg. The performance of compound **5** was best in minimizing the production of nitrate-N.

In conclusion, antifungal data of hydrazones revealed that compounds with a monosubstituted phenyl ring exhibit better activity than those with a disubstituted phenyl ring. Most of them were found to be potent insecticidal agents at a 0.1% dose against *S. litura*. Compound **29** (ED_{50} = 34.2−188.2 μg/mL), comprising a cyclohexyl ring, has emerged as a potent fungicide having insecticidal properties with mortality range of 70% against *S. litura*. The result obtained from bioassay indicates that this class of compounds can be utilized for the design of new molecules with good pesticidal activity. Thus, these molecules hold promise for further detailed bioefficacy study, especially against insect pests.

Supporting Information Available: MP, yield, IR, mass, and NMR data of synthesized compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

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Received for review November 25, 2009. Revised manuscript received January 19, 2010. Accepted January 19, 2010.