Some Furfural Derivatives as Nitrification Inhibitors

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Three series of furfural derivatives, namely *N*-*O*-furfural oxime ethers, furfural Schiff bases (furfurylidene anilines), and furfural chalcones, have been synthesized and evaluated for nitrification inhibition activity in laboratory incubation studies in typic Ustocrept soil. Furfural oxime ethers and furfural Schiff bases showed potential activity, but furfural chalcones were only mildly active. *N*-*O*-ethyl furfural oxime among the oxime ethers, and furfurylidine-4-chloroaniline among the furfural Schiff bases, performed the best. These two compounds showed more than 50% nitrification inhibition on the 45th day at 5% dose as compared to 73% inhibition by nitrapyrin. Activity of furfural oxime ethers decreased with an increase in carbon atoms in the *N*-*O*-alkyl side chain. Introduction of a chlorine atom in the phenyl ring of furfurylidene anilines increased the persistence of their activity. *N*-*O*-Ethyl furfural oxime and furfurylidine-4-chloroaniline coated urea performed at par with their application in solution form. Ethyl and *N*-*O*-isopropyl oxime, as well as chloro- and nitro-substituted Schiff bases, did not reveal any phytotoxicity (adverse effect on germination) on chickpea seeds (*Cicer arietinum*) even at the highest dose (40 ppm, soil basis).

Keywords: *Nitrification inhibitors; furfural oxime ethers; furfural Schiff bases; furfural chalcones; nitrapyrin*

INTRODUCTION

Nitrogen is the major nutrient limiting agricultural production in most soils. N-fertilizers have made a major contribution toward improving agricultural productivity the world over. The commonly used nitrogenous fertilizers, especially urea, suffer from the drawbacks of a low nitrogen use efficiency (NUE) and contributing toward environmental pollution. The NUE hardly exceeds 50% under most situations, and it drops below 30% in the case of lowland rice culture (*1*, *2*) The nitrification mediated by obligate chemolithotrophic bacteria (Family Nitrobacteraceae) seems to be the major reason for the low NUE and high nitrogen losses. The nitrate anion is more susceptible to leaching, thereby causing groundwater pollution (*3*). As a result of denitrification, nitrate is transformed into nitrous and nitric oxides, which have been implicated in global warming and depletion of the ozone layer. It has been established that the fertilizer nitrogen contributes 1.5 Teragram (Tg = 10^{12} g) N_2O-N yr^{-1} (*4*) and agriculture's contribution to global N_2O loading from the year 1986 to 2026 is likely to increase by 90% with increased N-fertilizer consumption (*5*, *6*). The use of nitrification inhibitors has been suggested to minimize these ill effects. Nitrapyrin, dicyandiamide, etridiazole, etc., are the commonly studied commercial nitrification inhibitors. The high cost of development and subsequent registration of effective nitrification inhibitors, the economics of their use, and the variable results obtained with their use in field conditions are the serious bottlenecks in their extensive use (*7*), underlining the need to develop simple, economical, efficient, and safe nitrification inhibitors.

Sahrawat et al (*8*) reported that simple furan derivatives such as furfural and furfural alcohol possessed good nitrification inhibitory properties. Among the various furan derivatives, 5-nitro-2-furfural oxime, furfural oxime, and furfural semicarbazone were the most effective (*9*). The nitro furan derivatives, however, led to an accumulation of nitrite, causing toxicity to *Nitrobacter sp*.

In this study twelve furfural derivatives have been synthesized and tested in the laboratory for their nitrification inhibitory property (Figure 1). Both the coating and solution applications of the inhibitors have been assessed. The promising compounds also have been evaluated for any adverse effect on germination of chickpea seeds.

MATERIALS AND METHODS

Chemicals and Reagents. These were procured from s. d. fiNE CHEM Ltd., Mumbai, India and used without further purification except furfural, which was freshly distilled each time before use. Nitrapyrin (90% purity) was obtained from Dow Elanco (Indianapolis, IN). Commercial-grade organic solvents were distilled before use.

Chromatography and Spectroscopy. Thin-layer chromatography (TLC) was performed on silica gel G plates, preactivated at 100 °C for 2 h. The ^{1}H NMR spectra were recorded on a Varian EM-360, 60-MHz instrument. Samples were dissolved in $CDCl_3$, and tetramethylsilane (TMS) was used as an internal standard. Chemical shifts are reported in δ values relative to TMS, and *J* values are expressed in Hertz. For colorimetric estimation, a Varian series 634 UV−Vis double-beam spectrophotometer was used.

Synthesis of Furfural Oximes. A mixture of freshly distilled furfural (0.2 mol, 19.2 g), hydroxylamine hydrochloride (0.1 mol, 13.9 g), and pyridine (20 mL) was refluxed in ethanol (100 mL) for 1 h on a water bath. Completion of the reaction was checked by TLC (hexane/acetone, 3:1). At the end of the reaction, ethanol was distilled off, and ice-cooled water (100 mL) was added. The precipitated furfural oxime (**I**) was

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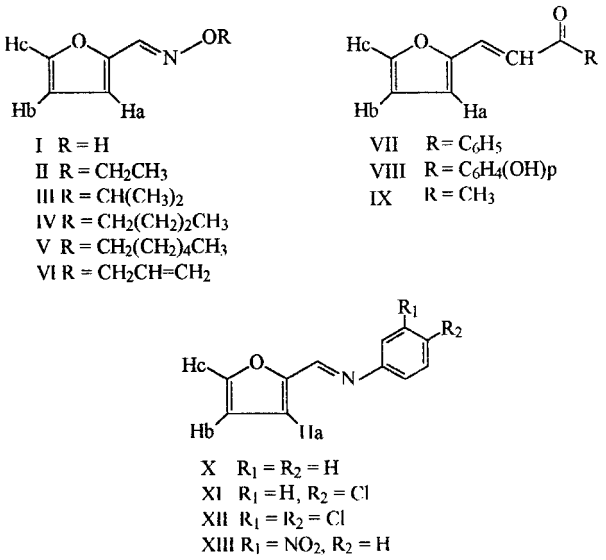

Figure 1. Generic structures of furfural derivatives.

filtered, dried, and recrystallized from ethanol as fluffy, colorless needles; mp. 86−87 °C; ^{1}H NMR ($CDCl_3$): δ 6.6 (m, $\mathbf{H}_b$), 7.6 (m, 2H, $\mathbf{H}_a$, $\mathbf{H}_c$), 8.2 (s, 1H, −C**H**=N−), 10.0 (s, 1H, broad, D_2O exchangeable O**H**); yield: 19 g (86%).

Synthesis of Furfural Oxime Ethers. A solution of furfural oxime (0.03 mol) and alkyl bromide (0.04 mol) in dry acetone (200 mL) was refluxed in the presence of dry potassium carbonate (10 g) for 48 h on a water bath. Completion of the reaction was checked by TLC (hexane/acetone, 3:1, visualizing reagent 2,4-diphenyl hydrazine). On completion of the reaction two pink spots were observed for the syn and anti isomers. The pink spots emerged fully after 15−20 min of the spray. After completion of the reaction, the solid potassium carbonate was filtered out, and the solvent was distilled off under reduced pressure. Ice-cooled water (100 mL) was then added, and the mixture was extracted with diethyl ether three times with 50 mL each of the solvent. The organic phase was dried over anhydrous sodium sulfate, and the solvent was distilled off to obtain a yellowish viscous liquid. This liquid was subjected to column chromatography over silica gel (60−120 mesh) using hexane/acetone (80:20) mixture as the eluant to yield yellowish viscous furfural oxime ethers. The test samples consisted of a mixture of two isomers.

N-O-Ethyl furfural oxime (**II**). R_f: 0.35, 0.78 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 1.6 (t, 3H, $-CH_2C\mathbf{H}_3$, J = 7 Hz), 4.1 (q, 2H, $-C\mathbf{H}_2CH_3$), 6.8 (dd, 1H, $\mathbf{H}_b$, *ortho* and *meta* coupled, J = 4 Hz), 7.7 (d, 1H, $\mathbf{H}_a$ *ortho* coupled, J = 4 Hz), 7.9 (s, 1H, −C**H**=N−), 8.0 (d, 1H, $\mathbf{H}_c$, *ortho* coupled, J = 3 Hz). Yield, 3.1 g (75%).

N-O-Isopropyl furfural oxime (**III**). R_f: 0.38, 0.80 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 1.6 (d, 6H, $-CH-(C\mathbf{H}_3)_2$, J = 6 Hz), 4.4 (heptet, 1H, $-C\mathbf{H}-(CH_3)_2$), 6.8 (dd, 1H, $\mathbf{H}_b$, *ortho* and *meta* coupled, J = 4 Hz), 7.8 (d, 1H, $\mathbf{H}_a$, *ortho* coupled, J = 4 Hz), 7.9 (s, 1H, −C**H**=N), 8.0 (d, 1H, $\mathbf{H}_c$, *ortho* coupled, J = 3 Hz). Yield, 3.4 g (75%).

N-O-Butyl furfural oxime (**IV**). R_f: 0.38, 0.83 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 1.2 (m, 3H, $-CH_2-(CH_2)_2C\mathbf{H}_3$), 1.6 (m, 2H, $-CH_2CH_2C\mathbf{H}_2CH_3$), 2.0 (m, 2H, $-CH_2C\mathbf{H}_2CH_2CH_3$), 4.0 (t, 2H, $-OC\mathbf{H}_2(CH_2)_2CH_3$, J = 6 Hz), 6.7 (dd, 1H, $\mathbf{H}_b$, *ortho* and *meta* coupled, 1 Hz), 7.7 (d, 1H, $\mathbf{H}_a$, *ortho* coupled, J = 4 Hz), 7.8 (s, 1H, −C**H**=N−), 7.9 (d, 1 H, $\mathbf{H}_c$ *ortho* coupled, J = 3 Hz). Yield, 3.8 g (76%).

N-O-Hexyl furfural oxime (**V**). R_f: 0.39, 0.84 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 1.0 (m, 3H, $-CH_2(CH_2)_4-C\mathbf{H}_3$), 1.4 (m, 8H, $-CH_2(C\mathbf{H}_2)_4CH_3$), 4.2 (m, 2H, $-C\mathbf{H}_2(CH_2)_4CH_3$), 6.7 (dd, 1H, $\mathbf{H}_b$), 7.4 (d, 1H, $\mathbf{H}_a$, *ortho* coupled, J = 4 Hz), 7.9−7.7 (m, 2H, $\mathbf{H}_c$, −C**H**=N). Yield, 4.3 g (74%).

N-O-Allyl furfural oxime (**VI**). R_f: 0.25, 0.76 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 4.8 (d, 2H, $-OC\mathbf{H}_2$, J = 4 Hz), 5.5 (m, 2H, $-CH_2CH=C\mathbf{H}_2$), 6.3 (m, 1H, $-CH_2C\mathbf{H}=CH_2$), 6.8 (dd, 1H, $\mathbf{H}_b$, *ortho* and *meta* coupled, J = 4 Hz), 6.9 (d, 1H, $\mathbf{H}_a$, *ortho* coupled, 4 Hz), 7.8 (d, 1H, $\mathbf{H}_c$), 7.9 (s, 1H, −C**H**=N−). Yield, 3.4 g (76%).

Synthesis of Furfural Chalcones. Three furfural chalcones, namely 3-(furan-1-yl)-1-phenyl-prop-2-en-1-one (**VII**), 3-(furan-1-yl)-1-4-hydroxyphenyl-prop-2-en-1-one (**VIII**), and 4-(furan-1-yl)-but-3-en-2-one (furfurylideneacetone, **IX**), were synthesized following the Claisen−Schmidt reaction (*10*). A methanolic solution of freshly distilled furfural was reacted with acetophenone, 4-hydroxyacetophenone ,and acetone in the presence of methanolic NaOH solution to obtain the desired compounds.

3-(Furan-1-yl)-1-phenyl-prop-2-en-1-one (**VII**. mp, 45−46 °C. R_f: 0.65 (hexane/acetone, 75:25); ^{1}H NMR ($CDCl_3$): δ 6.6 (dd, 1H, $\mathbf{H}_b$, J = 4 Hz), 6.9 (d, 1H, −CH=C**H**−CO, 4 Hz), 7.7 (m, 6H, 3 Ar−**H**, $\mathbf{H}_a$, $\mathbf{H}_c$, −C**H**=CH−CO), 8.3 (m, 2H, Ar−**H**). Yield, 15.8 g (80%).

3-(Furan-1-yl)-1-(4-hydroxy-phenyl)-prop-2-en-1-one (**VIII**), mp, 160 °. R_f: 0.32 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$/Acetone D_6): δ 6.6 (dd, 1H, $\mathbf{H}_b$, J = 4 Hz), 6.8 (d, 1H, −CH=C**H**−CO, 4 Hz), 7.1 (m, 2H, Ar−**H**), 7.5 (s, 1H, $\mathbf{H}_a$), 7.7 (m, 2H, C**H**=CH−CO, $\mathbf{H}_c$), 8.1(m, 2H, Ar−**H**), 9.0 (s, 1H, −O**H**, broad, D_2O exchangeable). Yield, 16.6 g (77%).

4-(Furan-1-yl)-but-3-en-2-one (furfurylideneacetone, **IX**). mp, 39 °C. R_f: 0.61 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 2.5 (s, 3H, −CO−C**H**$_3$), 6.6 (dd, 1H, $\mathbf{H}_b$, *ortho* and *meta* coupled, J = 4 Hz), 6.9 (d, 1H, CH=C**H**CO, J = 4 Hz), 7.4 (s, 1H, $\mathbf{H}_a$), 7.5−8.0 (m, 2H, −C**H**=CHCO, $\mathbf{H}_c$). Yield 8.2 g (60%).

Synthesis of Furfural-Schiff Bases. Furfural (0.1 mol, 9.6 g), substituted aniline (0.1 mol), and methanol (50 mL) were put in a beaker and heated gently on a water bath. The solution was then cooled in an ice bath. The solid that separated was filtered and recrystallized from methanol. Four Schiff bases, namely furfurylideneaniline (**X**), furfurylidene-4-chloroaniline (**XI**), furfurylidene-3,4-dichloroaniline (**XII**), and furfurylidene-3-nitro aniline (**XIII**) were synthesized, crystallized from methanol, and characterized spectroscopically.

Furfurylideneaniline (**X**). mp, 57 °C. R_f: 0.56 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 6.8 (dd, 1H, $\mathbf{H}_b$, *ortho* and *meta* coupled, J = 4 Hz.), 7.2 (d, 2H, Ar-**H**, *ortho* coupled, J = 8 Hz), 7.4−7.8 (m, 4H, 3Ar-**H**, $\mathbf{H}_a$), 7.9 (s, 1H, $\mathbf{H}_c$), 8.5 (s, 1H, −C**H**=N−). Yield 13.6 g (79.53%).

Furfurylidene-4-chloroaniline (**XI**). mp, 58 °C. R_f: 0.57 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 6.8 (dd, 1H, $\mathbf{H}_b$, *ortho* and *meta* coupled, J = 4 Hz), 7.2−7.8 (m, 5H, 4 Ar-**H**, $\mathbf{H}_a$), 7.9 (s, 1H, $\mathbf{H}_c$), 8.5 (s, 1H, −C**H**=N−). Yield 16.4 g (79.84%).

Furfurylidene-3,4-dichloroaniline (**XII**). mp, 43 °C. R_f: 0.57 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 6.8 (dd, 1H, $\mathbf{H}_b$, *ortho* and *meta* coupled, J = 4 Hz), 7.3 (m, 1H, Ar-**H**, *ortho* and *meta* coupled), 7.5 (m, 2H, 1Ar-**H**, $\mathbf{H}_a$), 7.9 (m, 2H, 1Ar-**H**, $\mathbf{H}_c$), 8.5 (s, 1H, −C**H**=N−). Yield 18.96 g (79%).

Furfurylidene-3-nitroaniline (**XIII**). mp, 56 °C. R_f: 0.45 (hexane/acetone, 75:25). ^{1}H NMR ($CDCl_3$): δ 6.8 (dd, 1H, $\mathbf{H}_b$, J = 4 Hz, *ortho* and *meta* coupled), 7.3 (d, $\mathbf{H}_a$, J = 3 Hz, *ortho* coupled), 7.8−6.5 (m, 5H, 4Ar-**H**, $\mathbf{H}_c$), 8.6 (s, 1H, −C**H**=N−). Yield 17.32 g (80.18%).

Nitrification Inhibition Study. *Test Soil.* The institute farm soil [sand 60.8%, clay 20.5%, silt 18.7%, organic carbon 0.5%; pH (soil/water, 1:2.5) 7.9; EC at 25 °C 0.35 dSm^{-1}; available N 55.72 kg ha^{-1}, nitrate-N 8.54 mg kg^{-1}, nitrite-N (traces), ammonium-N 3.20 mg kg^{-1}] was used for in vitro incubation studies.

Treatments. The test chemicals were evaluated for the nitrification inhibitory effect in laboratory incubation studies at 5, 10, 15, and 20% of applied urea-N along with the urea alone as control. The experiments were laid following randomized complete block design. A 200-g portion of soil was mixed thoroughly with the calculated amount of test chemical (2, 4, 6, 8 mg of test chemical, making respectively 5, 10, 15, and 20% of the applied 200 mg urea-N L^{-1} dose) followed by addition of 40 mg of urea-N in aqueous solution to provide 200 ppm urea-N in each treatment. Nitrapyrin (N-Serve) at 5% of applied urea-N was used as a standard. A control (200 ppm

urea-N alone) was simultaneously run. Distilled water was added to bring the soil moisture to one-third of the water-holding capacity of the soil. The treated soils were incubated in 500-mL beakers covered with muslin cloth at 28 °C and 98% RH in a BOD incubator. The moisture content of the incubated beakers was maintained by adding the required amount of distilled water every alternate day.

Sampling and Estimation of Ammonium, Nitrite, and Nitrate. Samples from the incubated soils were drawn after 15, 30, 45, and 60 days of incubation. For estimation of nitrate, a soil sample equivalent to 20 g of dry soil was withdrawn and extracted with 50 mL of distilled water (*11*), and for estimation of nitrite and ammonium a soil sample equivalent to 5 g of dry soil was withdrawn and extracted with 50 mL of KCl (*12*).

Ammonium and nitrite were estimated by following the indophenol blue method and modified Greiss-Ilosvay method (*12*), respectively. Nitrate was estimated by following the phenol disulfonic acid method (*11*).

The ammonium, nitrate, and nitrite contents were obtained from the standard curves and expressed in mg kg^{-1}. The nitrification rate and percent nitrification inhibition were calculated as per Sahrawat et al. (*13*) as follows:

$$\text{nitrification rate} = \frac{(NO_3^- + NO_2^-) - N}{(NH_4^+ + NO_3^- + NO_2^-) - N} \times 100$$

$$\% \text{ nitrification} = \frac{\text{nitrification rate in control} - \text{nitrification rate in sample}}{\text{nitrification rate in control}} \times 100$$

Coating versus Solution Application of Nitrification Inhibitors. *N-O*-Ethyl furfural oxime (**II**) and furylidine-4-chloroaniline (**XI**), the two most active furfural derivatives, were evaluated as coating on urea and as solution application. **II** was tested at 5 and 10% and **XI** at 2.5, 5, and 7.5% of the applied urea-N. For coating, urea was first sprayed with 2% coal tar in hexane as a binder, followed by spraying of the calculated amount of **II** and **XI** in dichloromethane. Upto 7.5% of **XI** could be coated on urea without any adverse effect on its physical form. Being more viscous, **II** could be coated up to 10% concentration by using 2% coal tar as binder, without any significant deterioration in its physical form though coating at 5% level of the inhibitor was the optimum.

For application as solution, 1 kg of air-dried soil was mixed with the calculated quantity of the test chemical in a volume of dichloromethane sufficient to ensure thorough mixing. After mixing, 200 mg of urea-N prills was added and mixed thoroughly. From the treated soil, twelve lots of 25 g each were taken in 50-mL beakers, water was maintained at one-third of the water-holding capacity, and the soil was incubated as before. Samples were periodically withdrawn and analyzed as described earlier.

Effect on Germination of Chickpea Seeds. Oxime ethers (**II** and **VI**) and furfural Schiff bases (**XI** and **XIII**) were evaluated for effects on the germination of chickpea seeds (*Cicer arietinum*) at 10 and 40 ppm levels (soil basis, equivalent to 5 and 20% of applied urea-N). The evaluation was done at room temperature in five replicates.

Statistical Treatment. The nitrification inhibition percentage data were statistically analyzed following the procedure laid out by Gomez and Gomez (*14*). The analysis of variance was computed and treatment means were compared by Duncan's multiple range test (DMRT) at 5% level.

RESULTS AND DISCUSSION

Nitrification Inhibitory Activity of Furfural Oxime Ethers. Results obtained in the in vitro soil incubation study are reported in Table 1. The oxime ethers in general have been found to be effective nitrification inhibitors. *N-O*-ethyl furfural oxime (**II**) was the most active. At 5 and 20% doses, it showed 67 and 80% nitrification inhibition (NI), respectively, on the 15th day. The corresponding NI values on the 45th day were 54 and 69%, respectively. When its dose was increased from 5 to 20%, the NI effect did not increase proportionately. As evident from the DMRT results, on all the four sampling days, each dose of **II** differed significantly from the other. The remaining ethers also showed a similar trend.

Nitrification inhibitory activity of **II** fell gradually with time up to 45th day. On the 15th day at 20% dose, 80% inhibition was observed; whereas on the 30th and 45th days, NI activity was 75 and 69%, respectively. After the 45th day, the activity fell sharply, and it

Table 1. Effect of Furfural Derivatives on Nitrification Inhibition

compd	dose (% urea-N)	nitrification inhibition (%)[a] 15th d	30th d	45th d	60th d
NS	5	83.14^{a}	76.55^{ab}	72.81^{a}	54.55^{a}
I	5	61.74^{qr}	49.44^{vwx}	32.61^{uv}	18.13^{w}
	10	65.31^{op}	55.06^{rs}	37.92^{st}	20.76^{uv}
	15	71.61^{ijkl}	60.04^{mnop}	42.71^{pq}	25.60^{opqr}
	20	73.76^{fghi}	62.17^{jkl}	44.74^{op}	26.42^{nopq}
II	5	67.04^{mno}	60.64^{lmno}	53.58^{l}	27.78^{mno}
	10	72.98^{ghijk}	68.88^{cdef}	59.15^{jk}	33.07^{hi}
	15	76.05^{cdef}	70.79^{c}	65.76^{def}	37.27^{fg}
	20	79.67^{b}	74.54^{b}	69.20^{bc}	41.80^{d}
III	5	66.90^{mno}	59.23^{mnopq}	51.45^{lm}	23.75^{qrs}
	10	69.85^{klm}	64.44^{ij}	58.02^{k}	32.12^{hij}
	15	74.97^{defgh}	69.32^{cdef}	62.88^{fgh}	36.51^{fg}
	20	79.16^{b}	71.47^{c}	66.41^{cde}	39.24^{def}
IV	5	65.08^{op}	58.18^{nopq}	49.41^{mn}	24.40^{pqrs}
	10	69.28^{lmn}	65.02^{hi}	54.00^{l}	29.49^{jklm}
	15	73.80^{fghi}	67.29^{fgh}	59.59^{ijk}	33.32^{hi}
	20	76.99^{bcde}	70.18^{cde}	62.41^{ghi}	36.57^{fg}
V	5	53.06^{tu}	48.83^{wx}	37.21^{t}	20.91^{tuv}
	10	59.55^{rs}	52.04^{tuv}	41.11^{qrs}	26.11^{opqr}
	15	64.43^{opq}	57.19^{pqr}	50.86^{lm}	32.18^{hij}
	20	69.15^{lmn}	65.82^{ghi}	59.00^{k}	34.70^{gh}
VI	5	67.18^{mno}	60.65^{lmno}	38.37^{rst}	18.83^{vw}
	10	72.83^{ghijk}	64.19^{ij}	40.85^{qrs}	20.78^{uv}
	15	75.47^{defg}	68.93^{cdef}	47.66^{no}	22.62^{stu}
	20	77.18^{bcd}	71.00^{c}	49.88^{mn}	27.13^{mnop}
VII	5	65.27^{op}	47.00^{x}	30.42^{vw}	15.62^{xy}
	10	70.41^{jkl}	50.49^{uvw}	33.68^{u}	22.21^{stu}
	15	74.99^{defgh}	58.90^{nopq}	41.28^{qr}	29.03^{klmn}
	20	77.64^{bcd}	62.02^{kjlm}	47.71^{no}	32.80^{hi}
VIII	5	50.12^{u}	36.91^{z}	26.83^{x}	9.73^{z}
	10	54.20^{t}	41.59^{y}	33.45^{uv}	13.73^{y}
	15	59.68^{rs}	46.83^{x}	39.48^{qrst}	17.41^{wx}
	20	62.27^{pqr}	52.65^{stu}	41.64^{qr}	26.09^{opqr}
IX	5	57.52^{s}	43.09^{y}	28.73^{wx}	13.59^{y}
	10	63.27^{pq}	50.30^{uvw}	32.68^{uv}	19.41^{vw}
	15	66.50^{no}	58.11^{opq}	39.49^{qrst}	24.20^{qrs}
	20	70.91^{ijkl}	61.11^{klmn}	45.22^{op}	30.52^{ijkl}
X	5	69.22^{lmn}	51.66^{tuvw}	30.61^{vw}	22.01^{stu}
	10	70.97^{ijkl}	56.92^{qr}	38.53^{rst}	28.89^{klmn}
	15	74.03^{efghi}	67.56^{efgh}	52.13^{lm}	36.97^{fg}
	20	76.01^{cdef}	69.33^{cdef}	64.57^{defg}	39.45^{def}
XI	5	72.80^{ghijk}	67.74^{defg}	59.37^{ijk}	32.06^{hij}
	10	78.62^{bc}	70.38^{cd}	63.36^{efgh}	37.03^{fg}
	15	82.89^{a}	75.52^{ab}	66.95^{cd}	47.87^{bc}
	20	84.59^{a}	77.36^{a}	70.04^{b}	49.22^{b}
XII	5	70.03^{klm}	60.38^{lmno}	51.21^{lm}	32.36^{hij}
	10	73.19^{fghij}	63.68^{ijk}	60.35^{hijk}	38.62^{ef}
	15	77.21^{bcd}	68.89^{cdef}	62.25^{ghij}	45.10^{c}
	20	79.18^{b}	71.17^{c}	66.38^{cde}	47.09^{bc}
XIII	5	72.00^{hijkl}	53.83^{st}	31.55^{uvw}	23.50^{rst}
	10	73.39^{fghij}	58.44^{nopq}	38.59^{rst}	30.69^{ijk}
	15	74.81^{defgh}	68.76^{cdef}	57.17^{k}	39.62^{def}
	20	79.28^{b}	70.84^{c}	65.02^{defg}	41.39^{de}

[a] The means are average of three replicates. Those followed by common letter show insignificant difference. Standard deviation (±) with mean of three replicates ranged from ±0.2 to ±1.3.

reached 42% on the 60th day. All the other doses of **II** as well as all the doses of **III** and **IV** behaved similarly. The reduction in the activity of these chemicals with passage of time was attributed to their loss, probably due to their metabolism by soil microorganisms, and is associated with the reestablishment of the population of the nitrifying bacteria.

N-*O*-Ethyl furfural oxime (**II**) was found to be the most effective, though statistically it was equal to the corresponding *N*-*O*-isopropyl derivative (**III**) at different doses and times. Among the five oxime ethers, *N*-*O*-hexyl furfural oxime (**V**) was least active. At lower doses (5 and 10%) the nitrification inhibitory effect of **V** started diminishing after the 30th day, whereas at higher doses (15 and 20%), the activity fell rapidly after the 45th day. Unlike the other compounds, the lower activity of **V** did not persist for longer time.

N-*O*-allyl furfural oxime (**VI**) showed considerable NI activity initially (15th day) but the activity decreased rapidly after the 30th day at all four doses. On the 15th, 30th, and 45th days, the NI activities at 5% dose were 67, 61, and 38%, respectively. The corresponding values for **II** were 67, 61, and 54%. Thus, initially there was no statistically significant difference between **II** and **VI** at the 15th and 30th days, but on the 45th day the difference was pronounced. **VI** did not show persistent activity, and this can be attributed to the allyl group which is more susceptible to degradation.

As compared to furfural oxime, the activity of oxime ethers was significantly higher at all the doses. Initially, the difference was less pronounced, but after the 15th day, it became pronounced. Thus, the greatest advantage of oxime ethers as compared to the parent oxime is the increased persistence of activity in soil.

The activity of **II** did not differ substantially with nitrapyrin at the initial stages, but with the passage of time, the difference increased. Though the activity was less compared to that of nitrapyrin, the inhibition of nitrification was still to the extent of over 50% at 5% dose on the 45th day. Similarly, the NI activity at higher doses (15 and 20%) of the corresponding isopropyl and butyl derivatives was comparable to that of the standard nitrification inhibitor nitrapyrin. Unlike these test chemicals, the activity of nitrapyrin persisted even after the 45th day. *N*-*O*-Allyl furfural oxime ether (**VI**) initially showed activity comparable with that of nitrapyrin, but with passage of time the activity decreased.

The structure activity relationships (SAR) study showed that NI activity of the furfural oxime ethers decreased with an increase in chain length of the alkyl group. Introduction of a double bond in the side chain showed high activity initially but it did not persist for long.

Nitrification Inhibitory Activity of Furfural Chalcones. Among the three test chalcones, 3-(furan-1-yl)-1-phenylprop-2-en-1-one (**VII**) showed the best performance (Table 1). On the 15th day it showed 67% NI activity at 5% dose and 78% activity at 20% dose. The corresponding values on the 45th day were 30 and 48%, respectively. Thus, **VII** showed initially good activity which could not be sustained for long. All the doses exhibited the same trend. Higher doses showed better activity but not a long lasting effect. All the four doses showed significant differences among themselves. At the highest dose (20%), **VII** showed less activity (78%) as compared to nitrapyrin (83%) and the difference widened with passage of time. The remaining two chalcones, **VIII** and **IX**, were, however, less active. Activity of all the three chalcones decreased quickly with time. Starting from the 15th day, the activity of the compounds showed substantial difference with nitrapyrin even at the highest dose (20%) of application. The same dose of the chalcones showed significant difference in activity on the 15th, 30th, and 60th days, whereas the difference in activity on the 45th day was not significant. Introduction of a hydroxy group in the phenyl ring significantly reduced the activity of these compounds. The chalcones as a class failed to show significant NI property.

Nitrification Inhibitory Activity of Furfural Schiff Bases. Among the Schiff bases, furfurylidine-4-chloroaniline (**XI**) outperformed all the other compounds, and it was followed closely by **XII**. At 5 and 20% doses, it showed 73 and 85% NI activity respectively on the 15th day. The activity at higher doses (15 and 20%) did not differ significantly from that of nitrapyrin (5%) on the 15th and 30th days and was comparable even up to the 60th day. The lower doses (5 and 10%) showed comparable activity with nitrapyrin up to 30th day. These doses inhibited 60% nitrification as compared to the control up to the 45th day. Like nitrapyrin, its activity decreased gradually up to the 45th day but decreased rapidly after 45 days. All the doses of **XI** followed this pattern. Furfurylidine-3,4-dichloroaniline (**XII**) at 15 and 20% doses showed excellent NI activity. On the 15th day, it showed 70% NI at 5% and 79% NI at 20% dose. This compound inhibited more than 50% nitrification up to the 45th day. Its 15 and 20% doses showed marginally inferior activity as compared to that of nitrapyrin. As with **XI**, there was no significant difference between the 15 and 20% doses on the 45th day. The 15% dose can thus be considered optimum for both monochloro- and dichloro-substituted Schiff bases. Furfurylidine aniline (**X**) and furfurylidine-3-nitroaniline (**XIII**) showed considerable nitrification inhibition property up to the 30th day, but with the passage of time the activity decreased gradually. The NI activity at the 5 and 20% doses of **XIII** on the 15th day was 72 and 79% and on 45th day was 32 and 65%, respectively. In case of **X**, on the 15th day NI activity for the 5 and 20% doses was 69 and 76%, respectively, whereas on the 45th day the values were 31 and 65%. Thus, the 20% dose of both the compounds showed greater persistence of activity up to the 45th day followed by the 15% dose. In the case of the 5 and 10% doses, the activity started diminishing rapidly from the 15th day onward. On the 15th day the difference of activity between these was insignificant, but with the passage of time it became significant.

Thus, in case of the Schiff bases, the nitrification inhibitory activity persisted for longer periods with an increase in the dose of the compound. However, the increase in NI activity, though statistically significant, was not of a high order. The introduction of a chlorine atom in the phenyl ring apparently increased the persistence as well as the activity. However, introduction of more than one chlorine reduced the activity. The activity of dichlorosubstituted Schiff base was significantly inferior to that of the monochloro derivative at most doses and sampling days except on the 60th day. It has been reported previously (*15*) that the NI activity increased with the number of chlorine substituents in

Table 2. Effect of Coated vs Solution Application of N-O-Ethyl Furfural Oxime (II) and Furfurylidine-4-Chloroaniline (XI) on Nitrification Inhibition

compd	dose (% urea-N)	appl. method	nitrification inhibition (%)[a] 15th d	30th d	45th d	60th d
II	5.0	coating	67.90^{d}	61.24^{c}	56.51^{c}	29.16^{d}
		solution	65.13^{d}	60.86^{c}	56.74^{c}	29.25^{d}
	10.0	coating	74.05^{bc}	68.84^{b}	59.97^{bc}	36.16^{bc}
		solution	72.43^{c}	68.51^{b}	59.56^{bc}	34.97^{bc}
XI	2.5	coating	55.63^{e}	41.40^{d}	30.97^{d}	21.64^{e}
		solution	53.22^{e}	39.03^{d}	31.34^{d}	21.85^{e}
	5.0	coating	73.60^{bc}	65.78^{b}	60.35^{bc}	34.47^{c}
		solution	72.43^{c}	67.78^{b}	59.77^{bc}	35.31^{bc}
	7.5	coating	75.61^{b}	67.61^{b}	62.39^{b}	38.45^{b}
		solution	76.43^{b}	68.98^{b}	62.13^{b}	37.45^{bc}
nitrapyrin	5.0	solution	82.13^{a}	75.59^{a}	67.55^{a}	53.37^{a}

[a] The means are averages of three replicates. Those followed by a common letter show insignificant difference.

the aniline ring. The present results do not corroborate this finding.

There was no significant difference in activity between furfurylidineaniline (**X**) and its nitro derivative **XIII** at most of the doses and periods, though in some cases (such as the 20% dose on 15th day) significantly better activity was recorded with **XIII**. The nitro group failed to improve the activity of the substituted Schiff base. The NI activity of nonchlorinated Schiff bases, though initially higher, did not persist for longer periods. Initially, all the Schiff bases showed similar activity at a given dose and time, but with the passage of time the difference in activity between the chlorinated and the nonchlorinated Schiff bases widened.

General Comparison of Activity of Furfural Derivatives. An appraisal of the NI values indicated that on the 15th day all the four Schiff bases, oxime ethers except *N*-*O*-hexyl furfuraloxime (**V**) and the chalcone, 3-(furan-1-yl)-1-phenylprop-2-en-1-one (**VII**), revealed similar activity. In general, the Schiff bases performed slightly better than the oxime ethers. On the 15th day, the Schiff bases were undoubtedly the best, followed closely by the oxime ethers. The chalcones, except **VII**, were the poor third. With the passage of time, the difference in activity at 5% dose of the chalcones, Schiff bases, and the oxime ethers widened. Overall, chlorosubstituted Schiff bases were the best among the test compounds followed by *N*-*O*-ethyl, isopropyl, and butyl oxime ethers.

When NI activity of the test compounds was compared at the 20% dose, it was observed that, except **V**, **VII**, and **IX**, the remaining compounds performed distinctly better and were comparable on the 15th day. The observations on the 45th day revealed that the chalcones did not have a long-lasting effect, whereas the Schiff bases showed generally persistent activity. The difference in NI activity of the Schiff bases and *N*-*O*-ethyl furfural oxime started widening from the 60th day, but up to the 45th day they were comparable. None of the treatments led to an accumulation of nitrite nitrogen.

Coating Compared to Solution Application. The nitrification inhibition data reported in Table 2 revealed that the coated products, on most of the sampling days and doses, showed activity statistically similar with that of their application as solution. Thus, under laboratory conditions, any of the methods can be used to apply the inhibitor. However, the coated products would be preferable for field applications because of the convenience in handling.

Table 3. Effect of Oxime Ethers (II and III) and Schiff Bases (XI and XIII) on the Germination of Chickpea Seeds

compd	dose (ppm, soil basis)	germination (%)[a] ± SD
control	−	82 ± 2.1
II	10	79 ± 1.3
	40	82 ± 2.5
III	10	81 ± 1.5
	40	82 ± 2.5
XI	10	81 ± 2.4
	40	80 ± 0.9
XIII	10	83 ± 2.4
	40	81 ± 1.8

[a] The means are averages of five replicates.

Germination of Chickpea Seeds. The most active products, namely **II**, **III**, **XI**, and **XIII**, did not adversely affect the germination of chickpea seeds, even at the highest dose (40 ppm, soil basis; Table 3). The test compounds, as well as the control, showed around 80% germination. The vigor of the seedlings was also similar.

CONCLUSION

Furfural oxime ethers and furfural Schiff bases have been found to possess potential as nitrification inhibitors, but the chalcones in general showed poor activity. Excellent activity of oxime ethers and Schiff bases has been attributed to the aldoximino moiety attached to the furan ring. Among the furfural oxime ethers, the *N*-*O*-ethyl derivative was the most active, followed by *N*-*O*-isopropyl, butyl, *n*-hexyl, and allyl furfural oxime. Activity of oxime ethers increased with an increase in dose but not proportionately. At the lower dose of 5%, the activity of these compounds, though considerable, was lower than that of nitrapyrin. Apparently, an increase in the number of carbon atoms in the alkyl chain of the oxime ethers reduced the nitrification activity of the compounds. Activity decreased with the introduction of a double bond in the alkyl group. Furfural oxime, an intermediate used in the synthesis of furfural oxime ethers, itself possessed good activity, but the activity was not long lasting. Among the Schiff bases, the chlorinated products showed considerable activity up to 45 days. At the 20% dose, monochloro-substituted Schiff base was more active than nitrapyrin up to the 30th day and was comparable to nitrapyrin up to the 60th day. Application of oxime ether and Schiff base coated urea failed to show improvement when compared with their application in solution form. These products also did not show any adverse effect on the germination of chickpea seeds. It can thus be concluded that there exists a potential for improving the nitrogen use efficiency of urea fertilizer and minimizing environmental pollution from nitrogenous fertilizers by developing nitrification inhibitory compounds from industrially cheap raw materials such as furfural. On the basis of SAR leads, more derivatives can be synthesized and tested. However, nitrapyrin is still by far the most active nitrification inhibitor.

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