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DISSIPATION OF NITRAPYRIN (NITRIFICATION INHIBITOR) IN SUBTROPICAL SOILS

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Abstract. Nitrapyrin (2-chloro-6-(trichloromethyl)pyridine) is a specific nitrification inhibitor, applied in soils for reducing the nitrification process of nitrogenous fertilizers. The overall effect of nitrapyrin is enhancing the efficiency of nitrogenous fertilizers in soils and also controlling environmental pollution in water by preventing nitrate leaching in soils. Dissipation of nitrapyrin was evaluated in subtropical soils at two fortification levels of 2 and 4 $\mu g \cdot g^{-1}$. The extraction of nitrapyrin was done by quick, easy, cheap, rugged and safe (QuEChERS) method and quantitative analysis – by high-performance liquid chromatography (HPLC). Nitrapyrin residues declined consistently with time in both types of soils and were not detectable ($<0.05\ \mu g \cdot g^{-1}$) on the 45th day at 2 $\mu g \cdot g^{-1}$ and on the 60th day at 4 $\mu g \cdot g^{-1}$ application rate. Dissipation of nitrapyrin occurred in a single phase with the persistence data fitting well to the first-order kinetics. The half-life of nitrapyrin was 9.6 and 9.9 d at 2 $\mu g \cdot g^{-1}$ and 16.1 d and 17.3 d at 4 $\mu g \cdot g^{-1}$ application rate in both types of soils. The results revealed higher persistence of nitrapyrin at higher concentration (4 $\mu g \cdot g^{-1}$) in both types of soils, probably because of high temperature and humidity in subtropical soils.

Keywords: nitrification inhibitor; degradation of nitrapyrin; first-order kinetics; persistence; half life; subtropical soils

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INTRODUCTION

Nitrification inhibitors (NIs) are a group of agrichemicals which prevent the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) without affecting subsequent oxidation of nitrite (NO_2^-) to nitrate (NO_3^-). They have potential to reduce the NO_2^- emmisions but their optimal dose depends on the deployment of integrated N fertilizer management system that increases yields and help to achieve reduction in the loss of N as N_2O (Burzaco *et al*. 2013). NIs are mixed with the fertilizers to increase the yield of crops as well as the efficiency of fertilzers by affecting other processes of nitrogen cycle in soils such as mineralization-immobilization, nitrous oxide production, ammonia volatization, etc. The studies on dissipation of NIs are of interest from the environmental standpoint because of the production of nitrates and nitrous oxide from the process of nitrification. Nitrous oxide (N_2O), though present in much lower concentration than carbon dioxide (CO_2), has a very high global warming potential (Forster *et al*. 2007).

Soil is made of polyanionic clays and silicates having a net anionic charge. Therefore, ammonium (NH_4^+) bonds tightly to the soil but nitrate ions (NO_3^-) do not because nitrate is more mobile and leaches into ground water supplies. Wildlife such as insects, freshwater fish and amphibians are sensitive to nitrates since they can cause death or lead to development of anomalies in affected species. Nitrification also contributes to formation of ground level ozone, acid rain and photochemical smog, change in species diversity and other undesirable processes. NIs, including commercial products like N-serve or nitrapyrin, dicyandiamide (Amberger 1989) and DMPP (3-, 4-dimethylpyrazolephosphate) (Zerulla *et al*. 2001), have been reported to inhibit the ammonia monooxygenase enzyme and prevent the transformation of NH^{4+} to NO_3^- implying specificity in their mode of action (Prasad 2009). Recently, Srivastava *et al.* (2016) reported the degradation of 2-amino-4-chloro-6-methyl pyrimidine (AM), a new nitrification inhibitor and discussed about its efficacy in subtropical soils of rice-wheat cropping systems.

Nitrapyrin (2-chloro-6-(trichloromethyl)pyridine), an active ingredient of N-Serve, is a compound proposed for use as a specific nitrification inhibitor. Its use on soil along with fertilizers containing ammonium ions holds microbial oxidation and subsequent leaching losses. Nitrapyrin (NP) is a bactericide that inhibits the activity of soil nitrifying bacteria *Nitrosomonas*, thereby limiting the first step of nitrification. NP inhibits the growth of bacteria rather than producing toxic effect on *Nitrosomonas* species activity. Applying NP with fertilizers can potentially increase crop production and provide better environmental quality by reducing nitrogen mobility in agricultural fields.

The persistence and mobility of a chemical which also include pesticides in soil are influenced by the properties of the chemical as well as by the soil environment, site conditions, weather, and application methods (Gavrilescu 2005).

The information on degradation of any chemical compound helps to predict its environmental behaviour (Laskowski *et al*. 1983). In the present study, the persistence of NP at two commonly recommended concentration levels has been evaluated to assess the duration of effectiveness of this inhibitor in soils of subtropical region.

MATERIALS AND METHODS

Two surface soil samples (0–15 cm) A and B were collected with the help of a spade from the fields of Crop Research Centre (CRC), Pantnagar. The soil samples were dried in shade and crushed with a wooden roller, passed through a 2-mm sieve and stored in plastic jars for carrying out dissipation studies. The laboratory experiments were conducted from March to May 2018 in Agro-Chemical Laboratory of Chemistry Department at G.B. Pant University, Pantnagar. The weekly meteorological data during the experimental period were also recorded for the purpose of degradation studies.

Analytical grade (2-chloro-6-(trichloromethyl)pyridine), nitrapyrin of 98% purity, was obtained from the firm Sigma-Aldrich. The other chemicals used in the study were of analytical and high-performance liquid chromatography (HPLC) grade procured from E. Merck (India) Ltd. Triple distilled water prepared in the laboratory by quartz distillation unit was used for preparing solutions. The processed soil samples A and B were analyzed for their general physiochemical properties by the recommended standard methods (Page *et al*. 1982).

Persistence studies of NP in soil were carried out in six plastic tubs of about thirty litres capacity by placing soil layers of known characteristics (5 cm depth) of one type (soil A) in three tubs and that of the other type (soil B) in the remaining three tubs. The soil layers were allowed to equilibrate for 24 h and thereafter a concentration of 2 and 4 $\mu g \cdot ml^{-1}$ of NP was sprayed on both the soils A and B in four tubs, and the remaining two tubs (with two different soils) were kept as control. Samples of soil (50 g) were drawn periodically from each tub (0 (2h), 1, 3, 7, 14, 28, 45, 60, 90, and 120 days) and the residue of NP was extracted in triplicate and analyzed as given below.

The extraction of NP from the soil samples was done using the QuEChERS (quick, easy, cheap, rugged and safe) method (Anastassiades *et al*. 2003**)** which is a streamlined approach for analytical chemists to examine residue in matrix. Ten grams of soil from each tub was taken in 50 mL centrifuge tubes and 10 mL of distilled water. Additionally, 4 g of anhydrous magnesium sulfate ($MgSO_4$) and 2 g of sodium chloride (NaCl) were added to it. The contents were vortexed for 2 min. After vortexing, 4 ml of acetonitrile (CH_3CN) was added to it. The mixture was again vortexed for 2 minutes and then centrifuged for 5 min at 3,000 rpm. After phase separation, the upper layer (organic layer) was taken and filtered through 0.45 µm PTFE (polytetrafluoroethylene) disc filter. Quantification of NP in soil was done by injecting 20 µl of the extract and determining the peak area. Quantification of NP was done by HPLC which was of Dionex make, Ultimate 3000 system equipped with the RP C-18 column (25 cm × 4.6 mm i.d.), degasser pump and ultraviolet–visible spectrophotometry (UV–VIs) detector at 271 nm using the mobile phase acetonitrile: water (80:20, v/v) at a flow rate of 1 mL·min^{-1}. Under these conditions, the retention time (Rt) of NP was 5.7 min. (Fig. 1).

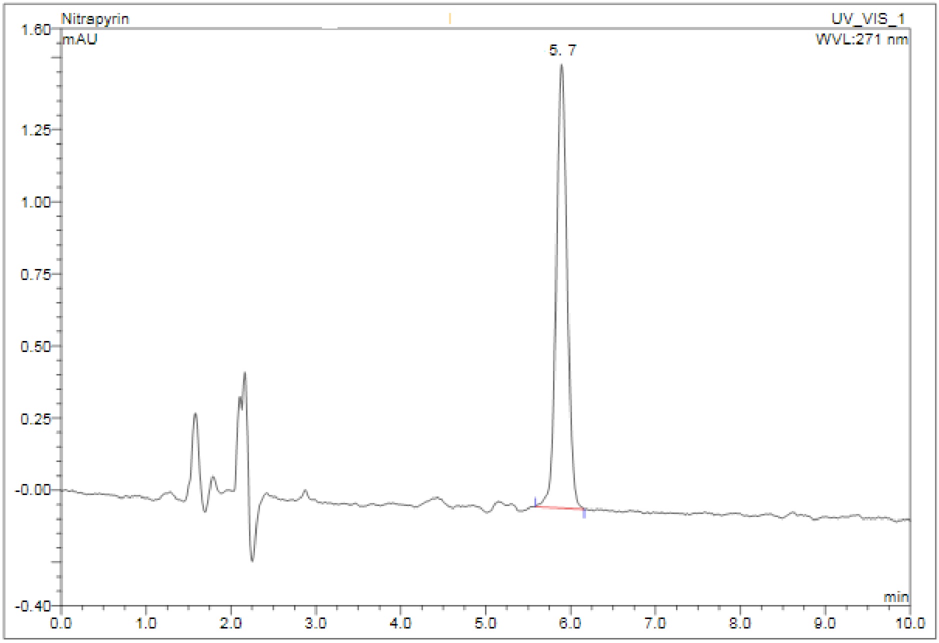

Fig. 1. Standard chromatogram of nitrapyrin (NP)

A linearity check was also performed for which a 100 µg·mL^{-1} solution of NP was prepared. It was diluted to 10 µg·mL^{-1} and thereafter serial dilutions of varying concentrations – 0.25, 0.5, 1, 2, 3, 4 and 5 µg·mL^{-1} – were prepared with HPLC grade acetonitrile. An aliquot of 20 µl was injected into the HPLC system every time for analysis. Peak area for each concentration was determined and a calibration curve was plotted between concentration and peak areas (Fig. 2). In order to check the efficiency of the method of extraction, recovery studies were performed. For this 10 g sample of each type soil was fortified with NP at 0.5, 1.0 µg·g^{-1} in duplicate. The extraction and quantification of the samples was done following the procedure described above. The limit of detection (LOD) of the instrument and limit of quantification (LOQ) on the basis of signal to noise ratio were also determined.

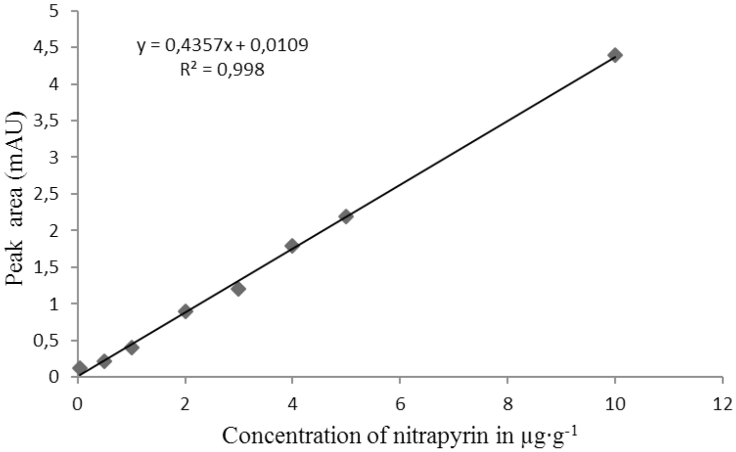

Fig. 2. Calibration curve of nitrapyrin at different concentrations

RESULTS AND DISCUSSION

The general properties of the soils are depicted in Table 1. The clayey soil sample was alkaline in nature with higher organic carbon content and soluble salts (higher electrical conductivity) as compared to sandy loam soil. The recoveries at two fortification levels, i.e. 0.5 and 1 µg·g^{-1} levels ranged between 83.5 ± 2.12 to 85.15 ± 1.62% in sandy clay loam and between 76 ± 2.82 to 78 ± 1.41% in clayey soil. The recovery was higher in sandy clay loam soil probably due to less adsorption because of lower clay content. During the experiment, the maximum and minimum temperatures varied in the range of 28.7–37.2°C and 10.7–23°C, respectively, with an average humidity of 65.2%. and average annual rainfall of about 10 mm. The LOD and LOQ values of nitrapyrin as determined by S/N ratio were found to be 0.05 and 0.16 µg·g^{-1}, respectively.

Table 1. Physicochemical properties of soil

Sl. No.	Properties	Soil type (depth 0–15 cm)	
		A	B
1	Textural class	Sandy clay loam	Clayey
2	pH	7.34	7.39
3	Electrical conductivity (dSm^{-1})	0.315	0.328
4	Organic carbon (%)	1.17	1.85

The persistence and dissipation data of nitrapyrin on different days is depicted in Table 2. The amount of residue recovered after the extraction process within two hours of nitrapyrin treatment (0 d) was considered as 100% and the amount of nitrapyrin residues at different intervals (0 to 60 d) were calculated taking that of 0 day as 100%. It is evident from the data in Table 2 that degradation of nitrapyrin residues was consistent in both types of soils at both application rates. NP could be detected up till 28 days at lower application rate and till 45 days at higher application rate in both soils A and B indicating a higher persistence at high application rate. Soils of this region (mollisols) are rich in carbon content and might be responsible for longer persistence of NP. McCarty and Bremner (1990) also reported that the persistence of NIs is higher in soils having a higher level of organic carbon.

The degradation data of NP was also fitted to first-order kinetic equation, $C = C_0 \cdot e^{-\lambda t}$ where C is amount of residue of nitrapyrin recovered from soil at time t, C_0 is amount residue recovered at $t = 0$, λ is degradation constant and t is time in days. The natural logarithm values of the amount of nitrapyrin residue (C) recovered at different time intervals for soil sample A and B were plotted against time as shown in Fig. 3a and 3b. The distribution of points in the graph produced single straight lines at both the concentrations indicating that dissipation of NP followed first-order kinetics.

Table 2. Persistence and dissipation percent of nitrapyrin (NP) in soil samples A and B at two concentrations

	Soil sample A		Soil sample B	
Days	2 $\mu g \cdot g^{-1}$	4 $\mu g \cdot g^{-1}$	2 $\mu g \cdot g^{-1}$	4 $\mu g \cdot g^{-1}$
0D	1.75 ± 0.02 (-)	3.79 ± 0.04 (-)	1.79 ± 0.03 (-)	3.81 ± 0.02 (-)
1D	1.51 ± 0.05 (13.8%)	3.20 ± 0.3 (15.6%)	1.52 ± 0.02 (15.1%)	3.27 ± 0.03 (14.2%)
3D	1.25 ± 0.01 (28.6%)	2.75 ± 0.02 (27.5%)	1.21 ± 0.04 (32.5%)	2.69 ± 0.01 (29.4%)
7D	0.90 ± 0.03 (48.6%)	2.38 ± 0.02 (37.3%)	0.92 ± 0.01 (48.7%)	2.43 ± 0.04 (36.3%)
14D	0.54 ± 0.02 (69.2%)	1.94 ± 0.01 (48.8%)	0.51 ± 0.02 (71.5%)	1.98 ± 0.03 (47.8%)
28D	0.22 ± 0.01 (87.4%)	0.97 ± 0.02 (74.5%)	0.24 ± 0.03 (86.6%)	0.89 ± 0.02 (76.5%)
45D	ND	0.50 ± 0.02 (86.8%)	ND	0.60 ± 0.01 (84.3%)
60D	ND	ND	ND	ND

Values in the parentheses show percent dissipation of the nitrapyrin (ND < 0.05 $\mu g \cdot g^{-1}$)

The computed values of degradation constant (λ), $T_{1/2}$ ($t_{1/2} = \log 2/k$), regression equation, and R^2 values for both the levels of fortification are summarized in Table 3. The statistically significant values of R^2 indicated that the dissipation data of NP conformed well to first-order kinetics. The calculated half-life values of NP in soil A and B were 9.6 and 9.9 days at 2 $\mu g \cdot g^{-1}$ soil application rate and 16.1 and 17.3 d at 4 $\mu g \cdot g^{-1}$ application rate, respectively.

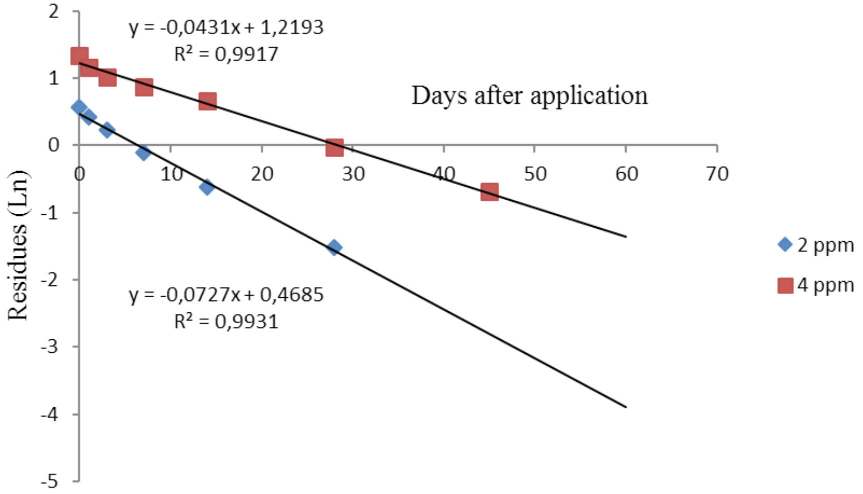

Fig. 3a. Plot between natural logarithm of nitrapyrin persistence versus time on days at different concentrations (2 μg·g^{-1} and 4 μg·g^{-1}) of soil sample A

In soil, the half-life of nitrapyrin ranges between 3 to 35 days, depending on soil type (CFS, 1985). Since the stability of a compound in two different soils is expressed in terms of half-life, it is clear from the data in Table 3 that nitrapyrin does not persist for long at lower concentration (2 μg·g^{-1}) but is more persistent at higher concentration (4 μg·g^{-1}) in both subtropical soil samples. The temperatures and humidity play an important role in nitrapyrin degradation and since both these are high in subtropical soils, the persistence of nitrapyrin is not

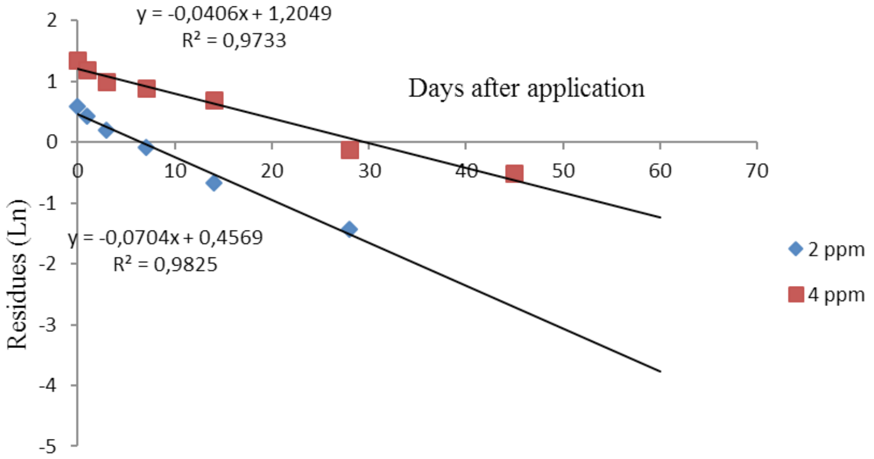

Fig. 3b. Plot between natural logarithm of nitrapyrin persistence versus time on days at different concentrations (2 μg·g^{-1} and 4 μg·g^{-1}) of soil sample B

Table 3. Determined values of degradation constant, half life ($t_{1/2}$) and coefficient of determination of nitrapyrin following first-order kinetics of degradation

Computed values	Soil sample A		Soil sample B	
	$2\ \mu g \cdot g^{-1}$	$4\ \mu g \cdot g^{-1}$	$2\ \mu g \cdot g^{-1}$	$4\ \mu g \cdot g^{-1}$
Degradation rate constant (λ)	0.072	0.043	0.070	0.040
Half life ($t_{1/2}$)	9.6 d	16.1 d	9.9 d	17.3 d
Coefficient of determination (R^2)	0.993	0.991	0.982	0.973
Regression equation	$y = -0.072x + 0.468$	$y = -0.043x + 1.219$	$y = -0.070x + 0.456$	$y = -0.040x + 1.204$

too high. These results are in accordance with the earlier reports in which it has been reported that the half-life of nitrapyrin is reduced with increasing temperatures (Touchton *et al.* 1979). Higher persistence of NP at higher application rate ($4\ \mu g \cdot g^{-1}$) can also be attributed to delayed nitrification in case of higher fortification rate. Similar results have been observed by Ali *et al.* (2008) when they did experiment with the nitrapyrin nitrification inhibitor at high soil temperature to locate its impact on nitrification. Since synchrony between soil mineral nitrogen (N) supply and crop N demand is important for optimal plant growth, management of nitrapyrin and pronitridine nitrification inhibitors with urea ammonium nitrate for winter wheat production has been reported (Habibullah *et al*. 2018). NP has also been reported to be effective in reducing nitrification and NO_3^- leaching losses in soil amended with manure from cattle feed and thus prevent ground water pollution (Luo *et al.* 2017). Moreover, NP addition has been found to mitigate nitrous oxide emissions (Liu *et al*. 2017), thus, playing an important role in preventing air pollution. Hence, it can be concluded that NP can prove to be an effective NI when mixed with fertilizers in subtropical soils in several aspects. However, further studies in fields need to be undertaken for confirmation of dissipation pattern and nitrate leaching due to the presence of NP in soil.

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