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Phototransformation of isoproturon in soil

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ABSTRACT

The present experiment was conducted with an objective to reveal the photochemical behaviour of isoproturon on different soil surfaces, *viz.* red, black and alluvial soil under sunlight. The half-life values of isoproturon on glass surface was found as 25.38 days. But on soil surfaces, the rate of photolysis was changed with half-life values of 20.76, 27.38 and 28.02 days under sunlight for red, black and alluvial soil, respectively. The slower reaction rate on the surfaces of black and alluvial soil was due to the quenching effect imparted by humic substances, which were absent on glass surface and less in red soil. The sunlight-irradiated extracts of isoproturon and its degradation products were analysed by LC-MS/MS using electrospray interfacing technique and the structures of six different photoproducts were characterised by their respective spectra as 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-hydroxymethylurea (I), 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-(*N*,*N*-dimethylcarbamoyl)urea (II), 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-(*N*-methylcarbamoyl)urea (III), 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-carbamoyl urea (IV), *N*-dimethyl-*N′*-methyl urea (V), *N*-dimethyl-*N′*-dimethyl urea (VI). The products were mainly formed through demethylation, ring oxidation and rearrangement. Thus, sunlight induced photodegradation may contribute in the dissipation of isoproturon in soil minimising the load of environmental hazards.

Key words: Dissipation, Isoproturon, Phototramsformation, Soil

Isoproturon, (3-(4-isopropylphenyl)-1,1-dimethyl urea) is a widely used herbicide for the weed management in wheat (*Triticum aestivum*). It kills broad-leaved weeds like *Phalaris minor* and *Avena ludoviciana* by inhibiting photosynthetic electron transport. The fate and toxicology of isoproturon are well documented. Its half-life ($T_{1/2}$) values in soil were observed to be 40 and 15 days, respectively in temperate and tropical climates (Kulshrestha and Muckerjee 1986). It undergoes slow hydrolysis in water with a half-life of 30 days (The WHO 2003). Due to its considerable persistency and mobility in soil, it carries the risk contaminating ground and surface water. The residues of isoproturon was detected in the ground water of different countries (Stuart *et al*. 2011). The degradation of this herbicide in soil may only minimise the risk of ground water contamination. In soil, it degrades mainly through microbial action generating non-toxic or less toxic degradation products (Sorensen and Aamand 2001, Sorensen *et al*. 2003). The rate of degradation of isoproturon by microbes may depend on the soil pH (Bending *et al*. 2003). Dureja *et al.* (1991) observed that the process of photolysis also plays a vital role on its degradation in water and identified 11 photoproducts in an aqueous solution irradiated under pure UV light within the range of 254 and 360 nm. The rate of photolysis of isoproturon is higher in organic solvent like methanol than that in water (de Saint-Laumer 1997). Thus in liquid phase, the role of photo-chemical reaction in the degradation of isoproturon was well investigated. Whereas no such thorough report on the photolysis of this herbicide in soil is available so far. Keeping this in view, an investigation was carried out on the photolysis of isoproturon under sunlight in different soils to find out the rate of degradation and to identify the various products thus formed.

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MATERIALS AND METHODS

Analytical grade isoproturon (99% purity) was procured from Sigma-Aldrich and used without further purification. Technical grade isoproturon (purity 96%) was procured from industry and purified further by silica gel-based column chromatography followed by repeated crystallization from cold ethanol until a constant melting point of 157^0C was achieved. Purity was checked by HPLC analysis comparing with analytical grade of it. Laboratory grade reagents and solvents were procured from Merck India, Mumbai. All solvents were dried and distilled before use. HPLC-grade solvents and reagents were used during

chromatographic and spectroscopic analysis. Deionized water was obtained from the Milli-Q SP Reagent water system (Millipore).

The alluvial, black and red soils used in this study were collected from the field of ICAR-IGFRI, Jhansi; ICAR-DWR, Jabalpur; and UAS, Bengaluru, respectively. The soils were air-dried and sieved through a 2 mm sieve. The physico-chemical properties of the soils (**Table 1**) were analysed by following standard procedures (Reeuwijk 2002).

Preparation of soil thin film: Thin films of soil were prepared from a suspension of 1 g soil in 2 ml of distilled water in the glass petridishes of 55 mm radius and air dried. All the surfaces, soil coated glass and non coated glass were coated uniformly with 1 ml of 10 µg/ml solutions of isoproturon in hexane.

Exposure of isoproturon on thin film under sunlight: For rate kinetic study, the thin films of isoproturon on soil and glass surfaces were irradiated under sunlight in cloudless days in the months of April and May between 9.30 am to 5.30 pm (8 h per day). The temperature during the irradiation varied at the test surface between 30 and 35^{0}C in sunlight. Samples from each surface were taken in triplicate at intervals of 0, 3, 6, 9, 15 and 21 days from initiation of exposure under sunlight. A set of 3 replications of each surface was kept under dark condition. For the photoproduct formation study, isoproturon was irradiated on the thin film of black soil under sunlight for 15 days following similar conditions maintained during the rate kinetic study.

Sample preparation and extraction procedure: Soil samples from each plate were scratched and collected in a 50 ml Erlenmeyer flask together with 15 ml acetone. It was shaken for 30 minutes and filtered in 100 ml round bottom flask using a 2µ filter paper. The process was repeated twice with the remaining soil in the Erlenmeyer flask and the filtrates were pooled together each time and concentrated under vacuum to a volume of 2 to 3 ml portion. It was then poured into a 25 ml separatory funnel containing 5 ml of 5% NaCl solution. The round bottom flask was washed with 10 ml of hexane that was added into the separatory funnel. It was shaken vigorously for about 2 minutes. The hexane layer was collected in round bottom flask. The process was repeated twice and the hexane layer was pooled together each time. The solution in hexane was then reduced to dryness at 45^{0}C and collected in spectroscopic grade methanol in water (60:40) of known volume. The samples from glass surface were directly taken in spectroscopic-grade methanol in water (60:40).

In order to standardise the extraction procedure a separate set of extraction and cleanup of samples was carried out with the isoproturon fortified sample of alluvial, black and red soil. Following the above mentioned extraction procedures, the recoveries of isoproturon were found to be 89.68 $\pm$ 5.57% from alluvial soil, 87.84 $\pm$ 3.38% from black soil, 92.22 $\pm$ 2.13% from red soil and 97.39 $\pm$ 1.09% from the glass surface.

For the isolation of photoproducts, the irradiated soil samples were extracted in ethyl acetate. The solvent was evaporated to dryness. It was then collected in spectroscopic grade acetonitrile, cleaned up through nylon-made membrane filters of 0.45 µm pore size and concentrated for mass analysis by LC-MS/MS.

Analysis of samples for rate kinetic study: For the rate kinetic study, the quantity of isoproturon extracted from each thin film was estimated on a Shimadzu LC 8A isocratic HPLC apparatus equipped with a photo diode array detector. The analysis of isoproturon was performed on a stainless steel column 250 mm long, 4.6 mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5 µm diameter (ODS, C18, 5µ, 250 × 4.6 mm id). The eluent was a methanol/water mixture in 60/40 (v/v) proportion. The flow rate of the solvent was maintained at 1 ml/minute and the eluent was monitored at the wavelength of 240 nm. A calibration was made for concentrations between 0.2 ppm and 1.4 ppm isoproturon with a linearity of R^2 = 0.9983.

Analysis of samples for the study on photoproduct formation: For the structural elucidation, products in the extract were characterized by LC-MS/MS. An API 3200 Qtrap mass spectrometer (AB Sykes Pvt. Ltd.) was used for the mass characterization of degraded products. Mass spectrometry analysis was performed with the electrospray ionization (ESI) in positive (5500 eV) mode for each sample. The nebulizer and heater gases were adjusted at 30 psi and 55 psi, respectively. The

Table 1. Physico-chemical properties of different soils

Soil	Order	Soil class	pH	EC (dS/m)	Clay (%)	Organic carbon (%)
Alluvial	Inceptisol	Clay loam	6.5	0.60	30.0	0.94
Black	Vertisol	Sandy clay loam	6.8	0.38	35.5	0.75
Red	Alfisol	Sandy loam	6.3	0.27	10.0	0.50

ion source temperature was set at 500°C. Each sample was injected by infusion technique at the rate of 10 µl/s.

UV-Visible spectra: UV-visible spectra of isoproturon in methanol was recorded on a Thermo-Fischer UV-VIS spectro-photometer using a quartz cuvette (1 cm path length).

Data analysis: Since the plots of natural log of isoproturon against time were assumed to be linear, a first-order equation was used to determine the rate constants (k) using the following equation:

$$N_t = N_0 e^{-kt}$$

Where, N_0 is the initial concentration of isoproturon, N_t concentration after a time t *i.e.* sampling time in days. The half-life was calculated by $t_{1/2} = 0.693/k$.

RESULTS AND DISCUSSION

Photodegradation of isoproturon

Isoproturon is reasonably photostable under sunlight condition. Its absorption maxima (λ_{max}) is 240 nm (**Figure 1**). The peak started from 210 nm ending at 255 nm exhibits the absorption due to the allowed π-π* transition and forbidden π-π* transition of the phenyl ring. The absorption of light at 275 nm is essentially n-ð* in character resulting from the urea group. The absorption spectrum of isoproturon in methanol overlaps the solar spectrum from 290 nm to 325 nm. The absorption of sunlight by isoproturon is, therefore, significant for the possibility of various photochemical reactions.

The rate of degradation of isoproturon was studied on the glass surface and soil surfaces. No significant change in the initial concentration was noticed in the control samples which were kept in the dark. Hence, the degradation observed in the irradiated samples must be attributed to a

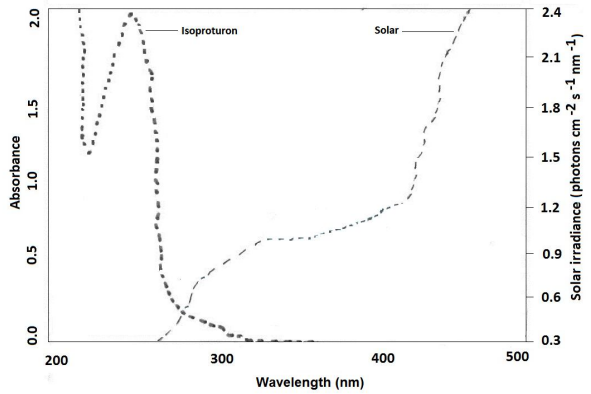

Figure 1. Comparison between isoproturon absorption spectrum and solar spectrum

Table 2. Rate kinetic equations and half-life values for the degradation of isoproturon on solid surfaces under the influence sunlight

Surface	Source of light	Rate constant (per day)	R^2	Half-life (days)
Glass	Sunlight	1.19×10^{-2}	0.8240	25.38
Red soil	Sunlight	1.45×10^{-2}	0.7770	20.76
Black soil	Sunlight	1.10×10^{-2}	0.8647	27.38
Alluvial soil	Sunlight	1.07×10^{-2}	0.9589	28.02

phototransformation process. Photolysis of isoproturon on all the surfaces followed first-order rate kinetic with significant regression coefficients (R^2) (**Table 2**). The glass surface provides an almost inert environment in which organic compound undergoes direct photolysis under irradiation. The half-life values of isoproturon on glass surface was 25.38 days. But on soil surfaces, values were 20.76, 27.38 and 28.02 days for red, black and alluvial soil, respectively (**Table 2**). Probably, the organic matter or humic substances present in black or alluvial soil acted as a photoquencher for isoproturon slowing down the rate of reaction.

Though isoproturon is reasonably photostable under sunlight, it still can undergo photolysis as it absorbs considerable amount of radiation in the UV-portion of sunlight. The degradation may also be possible due to the interaction between isoprturon and reactive species like singlet oxygen, hydroxyl radical, methyl radical, *etc*. which are formed due to the irradiation of humic substances present in soil (Hessler *et al*. 1996, Schmitt *et al*. 1995, Wenk *et al*. 2011, Remucal 2014). In the present experiment, photolysis of isoproturon on different surfaces, *viz*. glass, red soil, black soil and alluvial soil generated six major photoproducts, the structures of which were elucidated by mass spectra obtained from LC-MS/MS analysis and confimed by related literatures (**Figure 2**). The products are 3-(4-isopropyl-2/3-hydroxy-phenyl)-1-methyl-1-hydroxymethylurea (I), 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-(*N*,*N*-dimethylcarbamoyl)urea (II), 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-(*N*-methylcarbamoyl) urea (III), 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-carbamoyl urea (IV), *N*-dimethyl-*N′*-methyl urea (V), *N*-dimethyl-*N′*-dimethyl urea (VI) (**Figure 3**).

The product I was formed due to the hydroxylation at *N*-methyl group forming an intermediate OH-adduct of isoproturon, followed by the ring hydroxylation at either *o*- or *m*-position. Similar ring hydroxylation was also observed by Azizi *et al*. (2013) during the photolysis of isoproturon mediated by advanced oxidation processes. Halladja

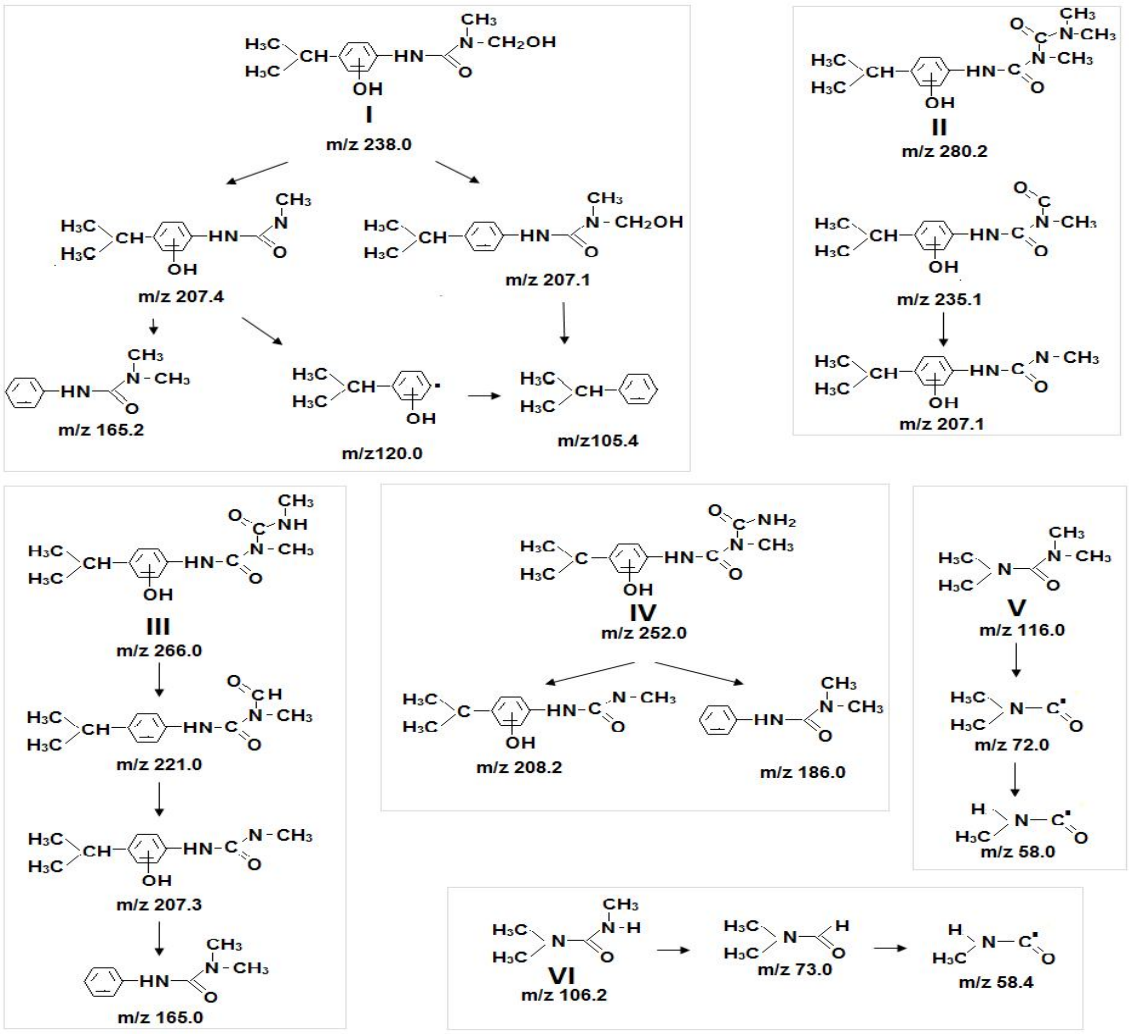

Figure 2. Mass fragmentation patterns of photo products formed during the photolysis of isoproturon on black soil surface under sunlight [I: 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-hydroxymethyl urea; II: 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-(*N*,*N*-dimethylcarbamoyl)urea; III: 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-(*N*-methylcarbamoyl)urea; IV: 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-carbamoyl urea; V: *N*-dimethyl-*N′*-methyl urea; VI: *N*-dimethyl-*N′*-dimethyl urea]

et al. (2007) also noticed the phenomenon of ring hydroxylation of another urea herbicide fluometuron during its photolysis in aqueous phase in presence of nitrate and fulvic acid, which on irradiation assisted in the generation of hydroxyl group. In our experiment, hydroxyl group containing chemical constituents present in humic substances are probably the source of active hydroxyl ions. During the irradiation process, homolysis of different bonds of isoproturon, *viz*. C(ring)-N, C(carbonyl)-N, C(methyl)-N led to the formation of different radicals, which in consequence reunited with each other forming different products. The product II was formed through such reunion of radicals followed by ring hydroxylation. The product V was also formed by a homolytic cleavage at the C(ring)-N, followed by the addition of one methyl radical. The fission between N-CH_3 of V followed by the addition of one hydrgen atom led to the formation of the product VI. Products III and IV were the results of the consecutive demethylation of II either through the homolytic cleavage of a methyl radical, or through the formation of hydroxyl methyl group followed by the formation of formyl group as stated by Sukul and Roy Chowdhury (1995).

Phototransformation of isoproturon on soil surfaces was not much influenced by any property of soil. The organic matter present in black and alluvial soil acted as a photo quencher or as photo screen to protect isoproturon from the direct photolysis. The organic or humic substances generated hydroxyl group and methyl radical on irradiation under sunlight. These reactive species chemically acted upon the isoproturon molecules transforming them into different products. Demethylated products and

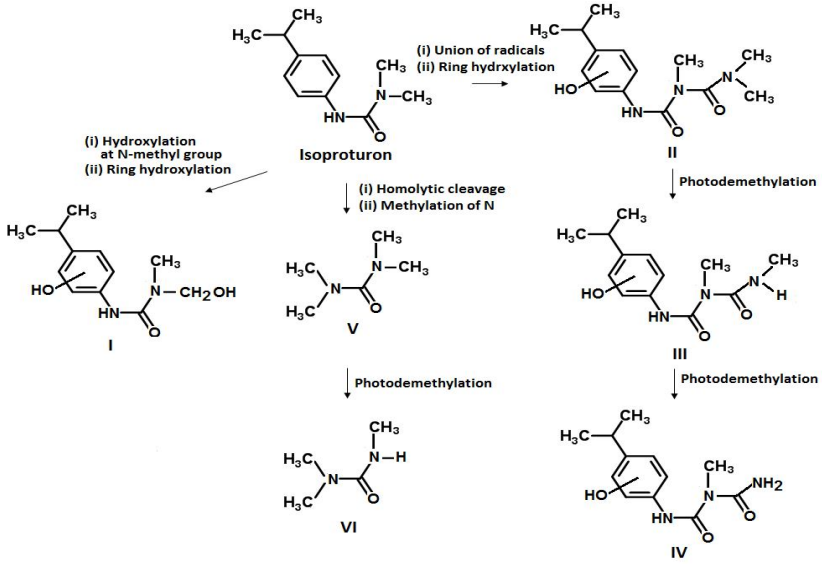

Figure 3. Pathways for the phototransformation of isoproturon in soil under sunlight [I: 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-hydroxymethyl urea; II: 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-(*N*,*N*-dimethylcarbamoyl)urea; III: 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-(*N*-methylcarbamoyl)urea; IV: 3-(4-isopropyl-2/3-hydroxyphenyl)-1-methyl-1-carbamoyl urea; V: *N*-dimethyl-*N′*-methyl urea; VI: *N*-dimethyl-*N′*-dimethyl urea]

aniline derivatives of isoproturon, which are generally formed during the photolytic process in aqueous phase or even in soil were not found in the present experiment. Terminal *N*-methyl substituted derivatives were formed (products II, III and IV), the toxicity of which are unknown.

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