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Full Length Article

Spatial Distribution of Isoproturon Dissipation in Varying Agricultural Lands and Characterization of an Isoproturon Degrading Bacterial Strain S29 from Genus *Sphingobium*

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Abstract

Isoproturon is a phenylurea herbicide had been extensively applied during wheat crop production in various countries including Pakistan. Understanding the fates of this herbicide within the agricultural fields and devising the strategies for its remediation is a topic of interest for the global scientific communities. The present study evaluates the variability of isoproturon dissipation within three agricultural fields having varying crop rotations *viz.* wheat-cotton, wheat-maize and wheat-rice rotations. The isoproturon dissipation in the selected fields was modelled using a modified Gompertz Model and different parameter including the maximum isoproturon dissipation (A) and the maximum rate of isoproturon dissipation (µm) were estimated. The modelled values showed that isoproturon dissipation was relatively higher in the loam soil under wheat-maize crop rotation followed by the sandy loam soil under wheat-cotton crop rotation as compared with the clay loam soil under wheat-rice crop rotation. Within the fields, the variability in isoproturon dissipation was found to be significantly linked with different soil parameters including pH, organic matter content, total organic carbon and the bacterial abundance. In order to further explore the isoproturon dissipation in these agricultural soils, an isoproturon degrading bacterial strain designated as *Sphingobium* spp. S29 was also isolated from the same soils. This strain had the potential not only to degrade isoproturon but also its known metabolites and other related phenylurea herbicides including diuron and chlorotoluron. Based on the findings of this study, it can be concluded that the targeted agricultural fields are adapted for isoproturon degradation due to repeated exposure to isoproturon and that the isoproturon degrading strain *Sphingobium* spp. S29 might be playing a significant role in dissipation of isoproturon in these soils. © 2019 Friends Science Publishers

Keywords: Isoproturon; Biodegradation; Spatial variability; Soil properties; Crop rotations; *Sphingobium*

Introduction

Isoproturon is a herbicide which belongs to phenylurea herbicides family. It is used for controlling a wide range of broadleaf weeds in a number of cereal crops including wheat and barley (Hussain *et al*., 2015). It is extensively used worldwide including China, France, United Kingdom and Pakistan (Nitchke and Schussler, 1998; Yin *et al*., 2008; Mahboob *et al*., 2011; Hussain *et al*., 2015). A number of studies have indicated the detection of isoproturon in several surface and ground water samples above the permissible limits (Nitchke and Schussler, 1998; Sorensen *et al*., 2003; Hussain *et al*., 2015). This detection is primarily linked not only with its repeated as well as intensive use in agricultural crops but also with the physicochemical properties it harbours. The data from ecotoxicological studies of isoproturon indicate that the presence of this herbicide in water resources is detrimental for animals and plants as well as for microbial communities and human beings because of this compound's and some of its metabolites' carcinogenic properties (Widenfalk *et al*., 2008; Yin *et al*., 2008; Vallotton *et al*., 2009; Dosnon-Olette *et al*., 2011; Hussain *et al*., 2015). Hence, in order to cope with the harms associated with the use of this herbicide, there is a dire need to limit its dispersion in different components of the environment by promoting its degradation in the environment through a thorough understanding of the processes and mechanisms which play a key role in determining its fate in agricultural lands.

In order to cope with isoproturon associated harms in agricultural systems, several researchers worldwide have focused their research on understanding the ways for reducing the exposure and dispersion of this herbicide after its application. Microbial biodegradation is considered as a

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key process for removal of this herbicide from the agricultural soils (Sorensen *et al*., 2003; El-Sebai *et al*., 2007; Hussain *et al*., 2011, 2015). Although a number of studies have been carried out to devise the strategies for bioremediation of the sites which are heavily contaminated with organic pollutants, very few studies were focused on understanding the processes involved in bioremediation of the sites which are diffusely contaminated with such organic compounds and pesticides. The diffused contamination of the soil sites resulting from the agricultural use of isoproturon also requires that the mechanisms and processes crucial for microbial biodegradation and reduction in its dispersion to different components of the environment should be elucidated.

During the recent two decades, numerous studies were conducted to evaluate the isoproturon dissipation in soils where this herbicide was continuously applied as well as in the soils of different geographical regions where soils exhibit variable physicochemical properties (Sorensen and Aamand, 2001; Sorensen *et al*., 2001; Walker *et al*., 2001; Bending *et al*., 2003; Sorensen and Aamand, 2003; El-Sebai *et al*., 2005, 2007; Hussain *et al*., 2013, 2015, 2017; Li *et al*., 2017). The most of these soils repeatedly exposed to isoproturon were found to have a good potential for degradation and dissipation of this herbicide. Moreover, the dissipation of isoproturon in these soils was primarily linked with the adaptation of the microbial communities for rapid degradation of isoproturon to use it as an extra source of carbon, nitrogen and energy (Sorensen and Aamand, 2001; Bending *et al*., 2003; Sorensen and Aamand, 2003; El-Sebai *et al*., 2005; Hussain *et al*., 2011, 2013, 2015; Yan *et al*., 2016; Hussain *et al*., 2017). It was also discovered that the isoproturon degradation in these soils was spatially variable not only from field to field but also at different points within the same field (Hussain *et al*., 2015). The study of this variability is important because it might be helpful in devising the remediation strategies for the agricultural fields under varying locations and with varying properties (Hussain *et al*., 2017). Moreover, it was discovered that degradation and dissipation of isoproturon in such repeatedly exposed adapted agricultural fields was linked with various biological and physicochemical characteristics of the soils including organic matter content, cation exchange capacity (CEC), microbial communities and pH (El-Sebai *et al*., 2005; Hussain *et al*., 2013, 2015, 2017). The existence of isoproturon degradation and mineralization has stimulated the research focused on isolation and characterization of isoproturon degrading microbial bioresources from such adapted soils. During the recent years, a few microbial strains including *Sphingomonas* spp. SH, *Sphingomonas* spp. SRS2, *Sphingobium* spp. YBL1, *Sphingobium* spp. YBL2 and *Sphingobium* spp. YBL3 have been isolated and characterized for isoproturon degradation (Sorensen *et al*., 2003; Sun *et al*., 2009; Hussain *et al*., 2011). Despite of a few reports on characterization of isoproturon degradation and mineralization in agricultural fields as well as on isolation of isoproturon degrading bacterial resources, it will be very important to further elucidate the mechanisms of isoproturon degradation by targeting not only some new agricultural fields which might have adapted for this phenomenon but also by isolating and characterizing new isoproturon degrading bacterial bioresources.

Isoproturon is also one of the major herbicides which are applied to control weeds in wheat crop in Pakistani agro-ecosystems. Despite its extensive use in Pakistani agriculture and that it has been detected in different components of the food chain (Mahboob *et al*., 2011), to the best of our knowledge, there is no study focused on understanding the mechanisms and processes involved in defining the fates, dissipation and remediation of isoproturon in the agricultural lands of Pakistan. Hence, this study has been conducted to evaluate the isoproturon degradation/dissipation in agricultural lands under wheat-rice, wheat-maize and wheat-cotton crop rotations which have been repeatedly exposed with this herbicide. This study also links the isoproturon dissipation with the prevailing physicochemical and biological properties of the soils from the selected agricultural fields. In addition to the dissipation kinetics of isoproturon in agricultural soils, this study also reports the isolation and characterization of a novel isoproturon degrading bacterial strain *Sphingobium* spp. S29. This is the first isoproturon degrading bacterial strain which has been isolated from Pakistani agricultural soil.

Materials and Methods

Soil Sampling and Characterization

Agricultural fields under wheat-rice, wheat-maize and wheat-cotton crop rotations were selected respectively from Jhang, Faisalabad and Sahiwal districts on the basis of a preliminary survey from the farmers. All the three fields had a history of more than ten years of isoproturon application. At first, composite samples from all three sampling fields were collected in March 2015 and analyzed for different physicochemical properties including pH, organic matter (OM) content, electrical conductivity (EC), texture, carbonates (CO_3^{2-}), bicarbonates (HCO_3^-), chlorides (Cl^-), calcium (Ca^{2+}) and CEC as shown in Table 1.

Texture of the soils was determined by estimating their particle size distributions using the Bouyoucos Hydrometer Method (Bouyoucos, 1962). For estimation of EC and pH, water : soil (5:1) suspensions were prepared. EC and pH were estimated using standardized EC and pH meter, respectively. The modified Walkley-Black method was used to estimate soil organic matter content (Walkley and Black, 1934; Rayment and Lyons, 2011). Sodium acetate method (1 *M* NaOAc, pH 7) was used to measure CEC of soil. Soil carbonates (CO_3^{2-}), bicarbonates (HCO_3^-), calcium (Ca^{2+}) and chlorides (Cl^-) were estimated according to USDA Handbook No.60 (US Salinity Laboratory Staff, 1954).

On the basis of varying crop rotations and textures as

Table 1: Physicochemical properties of the soils used for this study

Soil Properties	Values		
	Wheat-Cotton	Wheat-Maize	Wheat-Rice
Sand (%)	69	37	42
Silt (%)	24	47	31
Clay (%)	7	16	27
Textural Class	Sandy Loam	Loam	Clay Loam
EC (dS m^{-1})	0.51 ± 0.12	0.29 ± 0.07	0.69 ± 0.13
pH	8.35 ± 0.06	8.41 ± 0.08	8.52 ± 0.07
Organic Matter (%)	0.68 ± 0.14	0.77 ± 0.18	0.67 ± 0.11
CO_3^{2-} ($mmol_c$ L^{-1})	1.3 ± 0.21	2.2 ± 0.19	2.7 ± 0.24
HCO_3^- ($mmol_c$ L^{-1})	2.1 ± 0.18	4.9 ± 0.25	5.2 ± 0.13
Cl^- ($mmol_c$ L^{-1})	8.3 ± 1.2	9.6 ± 1.7	18.7 ± 1.6
Ca^{2+} ($mmol_c$ L^{-1})	3.4 ± 1.0	10.7 ± 1.9	15.6 ± 2.2
CEC ($cmol_c$ L^{-1})	7.15 ± 1.8	7.45 ± 1.1	7.76 ± 2.0

± indicates the standard deviation

well as a long-term isoproturon application history of the above three fields, the sampling for estimating the intra-field spatial variability in isoproturon degradation/dissipation was carried out from these fields in April 2015. For estimation of intra-field spatial variability in isoproturon dissipation, 54, 50 and 54 surface soil samples (0-15 cm) were obtained respectively from fields under wheat-rice, wheat-maize and wheat-cotton crop rotations. The sampling tools were sterilized with 70% ethanol prior to sample collection. The post sampling steps included performing sieving at 2 mm size and estimation of physicochemical characteristics of the soil samples.

Estimation of Physicochemical Properties of the Samples

All the soil samples collected from the above three agricultural fields were analysed for different physicochemical characteristics as described below.

The pH of the collected soil samples was estimated by making 1:5 soil : water suspensions and then using a standardized pH meter (HANNA Model 210). The EC of the collected soil samples was also estimated in 1:5 soil : water suspensions using a standardized EC meter (HANNA Model HI 8733). Cation exchange capacity of the collected soil samples was determined by following the sodium acetate method (1 *M* NaOAc, pH 7) as already done by Mehmood *et al*. (2018). Dissolved organic carbon (DOC) in the moist soil samples was estimated following the method of Ghani *et al.* (2013). For this purpose, 5.0 g of the soil was shifted into a 50 mL centrifuge tubes which was having 25 mL distilled water. It was allowed to shake horizontally at 150 rpm for 90 min. This mixture was centrifuged (10,000 × g for 5 min) and the supernatant was filtered using a Whatman Filter paper No. 42. The concentration of the organic carbon in the extract was estimated by following a modified Walkley and Black method (Walkley and Black, 1934; Jackson, 1962).

Total organic carbon (TOC) and organic matter (OM) contents in the soil samples were also estimated through modified Walkley and Black method (Walkley and Black, 1934; Jackson, 1962). For this purpose, 1.0 g fresh soil from each soil sample was digested by adding 10 mL of 1 N $K_2Cr_2O_7$ solution followed by continuous stirring along with addition of 20 mL of concentrated H_2SO_4. This mixture was allowed to stand for about 30 min. 200 mL distilled water was added into the mixtures and then 10 mL of concentrated orthophosphoric acid was added. This mixture was added with about 10-12 drops of diphenylamine indicator and titrated against 0.5 *M* ferrous ammonium sulphate till the appearance of green color as end point. Triplicate blanks without soil were also run in the same way. The TOC and the OM contents were estimated using the Equation 1 and 2, respectively.

$$TOC\ (\%) = 1.334 \times \frac{[V_{blank} - V_{sample}] \times 0.3 \times 10}{Wt \times V_{blank}} \quad \text{(Equation 1)}$$

$$OM\ (\%) = TOC\ (\%) \times 1.724 \qquad \text{(Equation 2)}$$

Where, V_{blank} is the volume (mL) of the ferrous ammonium sulphate required to titrate the blank, V_{sample} is the volume (mL) of the ferrous ammonium sulphate required to titrate the sample and Wt is the weight (g) of the air-dried soil.

Estimation of Biological Properties of the Samples

All the samples collected from the three agricultural fields were also analyzed for different biological properties including microbial biomass carbon, microbial biomass nitrogen and culturable heterotrophic bacteria as described below.

Microbial biomass carbon (MBC) in the soil samples was estimated following a fumigation-extraction technique as already reported by Vance *et al*. (1987). For this purpose, 10 g soil from each sample were incubated in a vacuumed desiccator to fumigate it for 48 h with alcohol-free chloroform. After fumigation, extraction was done by adding 40 mL of 30 m*M* K_2SO_4 and shaking it on an end-over-end shaker for 60 min. In parallel to fumigated soil sample, a non-fumigated was also processed in the same way and the total organic carbon in both types of samples was estimated following a modified Walkley and Black method (Walkley and Black, 1934; Jackson, 1962). The difference between the fumigated and non-fumigated carbon concentrations after an adjustment with an extraction factor was recorded as MBC. For estimation of microbial biomass nitrogen (MBN), the nitrogen in fumigated and non-fumigated samples were measured using a Kjeldahl apparatus. The difference between the fumigated and non-fumigated N concentrations was recorded as MBN.

The culturable heterotrophic microorganisms in each soil sample from the selected three agricultural fields were enumerated following the method described by Hussain *et al.* (2013). For this purpose, 10 g (equivalent dry weight) of the soil from each sample were suspended in 90 mL of the distilled sterilized water by mixing in a waring blender and serially diluted to 10^{-6}. 100 μL of the dilutions 10^{-4}, 10^{-5} and 10^{-6} were inoculated on NA/10 (beef extract 3.0 g L^{-1}, Peptone 5.0 g L^{-1}, Agar 16 g L^{-1}) media plates (n=3 per sample). The inoculated plates were incubated at 25°C for 10 days. The colonies appearing on the petri plates were enumerated using a digital colony counter.

Estimation of Isoproturon Dissipation in Soil Samples

For the estimation of potential for biodegradation/dissipation of isoproturon in agricultural fields, 400 g equivalent dry weight of each soil sample were treated with analytical grade isoproturon (15 mg kg^{-1} of the soil) and thoroughly mixed to make it homogeneous. The treated soils were transferred to sterile polypropylene containers and incubated at 25°C for 90 days. Throughout this experiment, the humidity of the soil samples was maintained to 80% of the water holding capacity by adding necessary amount of sterile distilled water and soils (20 g) were sampled after 5, 15, 30, 45, 60 and 90 days of incubation. Isoproturon residues remaining in the soil samples were extracted with methanol and analyzed using high performance liquid chromatography (HPLC) equipped with a 25 cm long C18 column (Varian), a UV detector at 243 nm and an acetonitrile/water (75/25, v/v) mobile phase flowing at rate of 1 mL min^{-1} as already described by Hussain *et al.* (2013). The isoproturon dissipation/degradation in the soil samples was calculated using the following formula (Equation 3).

$$Dissipation\ (\%) = \frac{(A_i - A_t)}{A_i} \times 100 \qquad \text{(Equation 3)}$$

Where, A_i is the area of peak of the sample at initial time (0 days) and A_t is the area of peak of the sample at specific time.

The biodegradation/dissipation of isoproturon in all samples of the three sets of soils were modeled using a modified Gomperz model (Zwietering *et al.*, 1990) to calculate the values of maximum (%) isoproturon dissipation (A), maximum rate (%.day^{-1}) of isoproturon dissipation (μm) and days of lag period (λ).

Isolation and Identification of Isoproturon Degrading Bacterial Strain

Isolation of the isoproturon degrading bacterial strain was done from the soil showing maximum dissipation of isoproturon through enrichment culture technique using a mineral salt (MS) medium as already reported by Hussain *et al.* (2011). Briefly, 10 g of the soil was inoculated in 90 mL of freshly prepared MS medium having 50 mg L^{-1} of isoproturon. The flasks (Control + sample) were incubated at 30°C under shaking (150 rpm). The aliquots were drawn at different time intervals and analyzed for remaining isoproturon in the medium following HPLC analysis as already reported by Hussain *et al.* (2011). When more than 70% of the initially added isoproturon was degraded, 10 mL of the slurry was inoculated in 90 mL of freshly prepared MS medium having 50 mg L^{-1} of isoproturon and processed in the similar way. After five such cycles of enrichment, 100 μL of each of 10^{-4}, 10^{-5} and 10^{-6} dilutions of the slurry were inoculated in MS agar media plates having 250 mg L^{-1} of isoproturon. The colonies appearing on the media plates were purified through repeated streaking and separately tested for degradation of isoproturon in MS media. The bacterial colony (S29) showing the potential for degradation of isoproturon was further purified and identified through amplification and sequencing of its 16S rRNA following the procedure already described by Hussain *et al.* (2011). The phylogenetic analysis of 16S rRNA of the isolate S29 was carried out using the Clustal X software as already reported by Hussain *et al.* (2011).

Characterization of Degrading Capabilities of the Strain S29

The strain S29 was evaluated for its potential to degrade different phenylurea herbicides including isoproturon, diuron, cholotoluron, linuron and monolinuron in MS media. For this purpose, 50 mg L^{-1} each of the phenylurea herbicide was added in separate MS media flasks and the cells of the strain S29 grown in MS isoproturon medium were added to develop an optical density (OD600) of 0.5 using an optical density meter. Triplicates of the flasks along with un-inoculated controls were incubated at 30°C under shaking (150 rpm). Aliquots were drawn at different time intervals (0, 2, 4, 10, 22, 28, 34, 47, 52 & 58 h) over the incubation period and analyzed for the respective phenylurea herbicides through HPLC analyses (Hussain *et al*., 2011). The strain S29 was also evaluated for its potential to degrade different known metabolites of isoproturon including monodemethylisoproturon, didemethylisoproturon and 4-isopropyl aniline in the MS medium following the same procedure as described for degradation of different phenylurea herbicides in above lines.

Statistical Analyses

The means values of the physicochemical, biological and modelled isoproturon dissipation parameters (A, μm and λ) of the soil samples from three agricultural fields were statistically compared through analysis of variance (ANOVA) using the SigmaPlot Software (14.0). The coefficient of correlation between the modelled isoproturon dissipation parameters (A, μm and λ) and the physicochemical and biological parameters of the soil samples were also estimated using the SigmaPlot Software (14.0). The correlation matrices between the physicochemical, biological and modelled isoproturon dissipation parameters of the soil samples from three agricultural fields were drawn using the statistical tools of R (version 3.4.3). The graphical analysis was performed using R-Package.

Results

Characterization of Physicochemical Parameters in the Selected Three Agricultural Fields

Descriptive statistics including minimum/maximum values, mean/median, standard deviation (SD) and coefficient of variation (CV) for the physicochemical parameters (cation exchange capacity, electrical conductivity, pH, organic matter content, total & dissolved organic carbon) of the 54 sub-site soil samples from the agricultural field under wheat-cotton rotation, 50 sub-site soil samples from the agricultural field under wheat-maize rotation and 54 sub-site soil samples from the agricultural field under wheat-rice rotation have been presented in Table 2. The values of all the physicochemical parameters were found to be considerably different not only from field to field but also at different points within each field.

The value of pH within the agricultural field under wheat-cotton rotation was found to vary between 8.11 to 8.88 with a CV value of only 2.31% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the pH was found to vary from 7.99 to 8.93 with a CV value of 2.17%. Similarly, a low CV value of 3.06% was observed within the agricultural field under wheat-rice crop rotation with the pH values ranging from 8.21 to 9.17. The analysis of variance (ANOVA) indicated that mean (8.57) pH values within the agricultural field under wheat-rice crop rotation was significantly higher than mean pH of fields under wheat-cotton (8.40) and wheats-maize (8.37) crop rotations which were statistically at par with each other.

The electrical conductivity (EC) of the soil samples within the agricultural field under wheat-cotton rotation was found to vary between 0.22 to 0.77 dS m^{-1} with a CV value of 34.09% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the EC was found to vary from 0.21 to 0.30 dS m^{-1} with a CV value of 8.92%. However, among the selected three agricultural fields, the highest of CV (62.63%) was observed within the agricultural field under wheat-rice crop rotation with the EC values between 0.18 and 2.23 dS m^{-1}. The statistical analysis through analysis of variance indicated that the mean (0.75 dS m^{-1}) of the EC values within the agricultural field under wheat-rice crop rotation was significantly higher than mean EC values of fields under wheat-cotton (0.44 dS m^{-1}) and wheats-maize (0.24 dS m^{-1}) crop rotations. The mean value of EC of the soil samples from the field under wheat-cotton rotation (0.44 dS m^{-1}) was also significantly higher as compared to the mean EC of the samples from the agricultural field under wheats-maize (0.24 dS m^{-1}) crop rotation.

The soil CEC of samples within the agricultural field under wheat-cotton rotation was found to vary between 6.33 to 9.17 $cmol_c$ kg^{-1} having 9.61% CV value of (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the CEC was found to vary from 6.39 to 7.97 $cmol_c$ kg^{-1} with a CV value of 5.98%. Similarly, a CV value of 5.12% was observed for the agricultural field under wheat-rice crop rotation with the CEC values ranging from 6.80 to 8.30 $cmol_c$ kg^{-1}. The statistical analysis through analysis of variance (ANOVA) indicated that the mean (7.80 $cmol_c$ kg^{-1}) of the CEC values within the agricultural field under wheat-cotton crop rotation was significantly higher as compared to the mean values of CEC for the fields under wheat-maize (7.23 $cmol_c$ kg^{-1}) and wheats-rice (7.58 $cmol_c$ kg^{-1}) crop rotations. The mean value of CEC of the soil samples from the field under wheat-rice rotation (7.58 $cmol_c$ kg^{-1}) was also significantly higher as compared to the mean CEC of the samples from the agricultural field under wheats-maize (7.23 $cmol_c$ kg^{-1}) crop rotation.

The dissolved organic carbon (DOC) of the soil samples within the agricultural field under wheat-cotton rotation was found to vary between 39.07 to 703.34 mg kg^{-1} having 74.65% CV (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the DOC was found to vary from 52.85 to 408.07 mg kg^{-1} with a CV value of 41.05%. However, among the selected three agricultural fields, the highest CV value (84.58%) was observed for the agricultural field under wheat-rice crop rotation with the DOC values ranging from 12.32 to 789.33 mg kg^{-1}. The statistical analysis through analysis of variance (ANOVA) indicated that the mean (123.66 mg kg^{-1}) of the DOC values within the agricultural field under wheat-cotton crop rotation was significantly lower as compared to the mean values of DOC for the agricultural fields under wheat-maize (219.15 mg kg^{-1}) and wheats-rice (217.25 mg kg^{-1}) crop rotations which were both statistically at par with each other.

Total organic carbon (TOC) of agricultural field under wheat-cotton rotation was found to vary between 0.18 to 0.56% with a CV value of 22.52% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the TOC was found to vary from 0.31 to 0.58% with a CV value of 12.71%. Similarly, a CV value of 23.72% was observed for the agricultural field under wheat-rice crop rotation with the TOC values ranging from 0.21 to 0.58%. The statistical analysis through analysis of variance indicated that the mean (0.47%) of the TOC values within the agricultural field under wheat-maize crop rotation was significantly higher than that of fields under wheat-cotton (0.43%) and wheats-rice (0.38%) crop rotations. The mean value of TOC of the soil samples from the field under wheat-cotton rotation (0.43%) was also significantly higher as compared to the mean TOC of the samples from the agricultural field under wheats-rice (0.38%) crop rotation.

The OM content in agricultural field under wheat-cotton rotation was found to vary between 0.31 to 0.97% with a CV value of 22.52% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the OM was found to vary from 0.53 to 0.99% with a CV value of 12.71%.

Similarly, a CV value of 23.72% was observed for the agricultural field under wheat-rice crop rotation with the OM values ranging from 0.36 to 1.0%. The statistical analysis through analysis of variance (ANOVA) indicated that the mean (0.80%) of the OM content values within the agricultural field under wheat-maize crop rotation was significantly higher than fields under wheat-cotton (0.73%)

Table 2: Descriptive analyses of physicochemical parameters [pH, electrical conductivity (EC), cation exchange capacity (CEC), dissolved organic carbon (DOC), total organic carbon (TOC), organic matter (OM)] and biological parameters [microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) number of cultivable bacteria (cfu), maximum percentage of isoproturon dissipation (A), maximum rate of isoproturon dissipation (μm), lag phase (λ)] determined from the soil samples collected from three varying fields (54 from wheat-cotton, 50 from wheat-maize, 54 from wheat-rice field)

	Sampling Field	Min	Max	Mean	Median	SD	CV
pH	Wheat-Cotton	8.11	8.88	8.40 **b**	8.38	0.19	2.31
	Wheat-Maize	7.99	8.93	8.37 **b**	8.39	0.18	2.17
	Wheat-Rice	8.21	9.17	8.57 **a**	8.52	0.26	3.06
EC (dS m^{-1})	Wheat-Cotton	0.22	0.77	0.44 **b**	0.39	0.15	34.09
	Wheat-Maize	0.21	0.30	0.24 **c**	0.24	0.02	8.92
	Wheat-Rice	0.18	2.23	0.75 **a**	0.63	0.47	62.63
CEC (cmolc kg^{-1})	Wheat-Cotton	6.33	9.17	7.80 **a**	7.87	0.75	9.61
	Wheat-Maize	6.39	7.97	7.23 **c**	7.21	0.43	5.98
	Wheat-Rice	6.80	8.30	7.58 **b**	7.60	0.39	5.12
DOC (mg kg^{-1})	Wheat-Cotton	39.07	703.34	123.66 **b**	106.89	92.30	74.65
	Wheat-Maize	52.85	408.07	219.15 **a**	216.22	89.97	41.05
	Wheat-Rice	12.32	789.33	217.25 **a**	166.24	183.75	84.58
TOC (%)	Wheat-Cotton	0.18	0.56	0.43 **b**	0.44	0.10	22.52
	Wheat-Maize	0.31	0.58	0.47 **a**	0.46	0.06	12.71
	Wheat-Rice	0.21	0.58	0.38 **c**	0.37	0.09	23.72
OM (%)	Wheat-Cotton	0.31	0.97	0.73 **b**	0.75	0.17	22.52
	Wheat-Maize	0.53	0.99	0.80 **a**	0.80	0.10	12.71
	Wheat-Rice	0.36	1.00	0.65 **c**	0.63	0.15	23.72
MBC (mg kg^{-1})	Wheat-Cotton	16.5	237.6	110.9 **b**	111.5	61.9	55.8
	Wheat-Maize	47.3	232.5	120.2 **b**	116.1	37.3	31.0
	Wheat-Rice	20.0	465.1	153.5 **a**	124.4	97.8	63.7
MBN (mg kg^{-1})	Wheat-Cotton	1.90	24.10	11.69 **a**	11.40	6.32	54.09
	Wheat-Maize	4.97	20.70	11.77 **a**	11.74	3.30	28.02
	Wheat-Rice	1.90	42.70	13.96 **a**	11.40	8.99	64.43
Culturable bacteria (cfu g^{-1})	Wheat-Cotton	1.93E+07	9.57E+07	5.92E+07**b**	5.90E+07	1.88E+07	31.80
	Wheat-Maize	2.70E+07	1.03E+08	6.70E+07**a**	6.65E+07	1.90E+07	28.38
	Wheat-Rice	2.34E+07	8.85E+07	5.11E+07**a**	5.05E+07	1.76E+07	34.41
A (%)	Wheat-Cotton	13.73	73.76	52.45 **b**	54.50	13.28	25.32
	Wheat-Maize	23.96	79.88	58.79 **a**	58.92	11.08	18.85
	Wheat-Rice	13.48	71.11	49.75 **b**	51.99	12.39	24.90
μm (%.day^{-1})	Wheat-Cotton	0.50	2.32	1.64 **a**	1.80	0.51	31.16
	Wheat-Maize	0.40	2.98	1.62 **a**	1.68	0.55	34.10
	Wheat-Rice	0.29	1.62	1.15 **b**	1.20	0.33	29.13
Λ (days)	Wheat-Cotton	2.47	14.37	7.01 **a**	6.82	2.13	30.32
	Wheat-Maize	1.30	17.17	5.34 **b**	4.40	2.70	50.58
	Wheat-Rice	0.10	14.69	5.85 **b**	5.25	2.71	46.33

Min= minimum; Max= maximum; SD= standard deviation; CV= coefficient of variation

and wheats-rice (0.65%) crop rotations. The mean value of OM content of the soil samples from the field under wheat-cotton rotation (0.73%) was also significantly higher as compared to the mean OM content of the samples from the agricultural field under wheats-rice (0.65%) crop rotation.

Characterization of Biological Parameters in the Selected Three Agricultural Fields

Descriptive statistics including minimum/maximum values, mean/median, SD and CV for the biological parameters [culturable heterotrophic bacteria, microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC)] of the 54 sub-site soil samples from the agricultural field under wheat-cotton rotation, 50 sub-site soil samples from the agricultural field under wheat-maize rotation and 54 sub-site soil samples from the agricultural field under wheat-rice rotation have been presented in Table 2. The values of all the biological parameters were found to be considerably different not only from field to field but also at different points within each field.

The microbial biomass carbon (MBC) of the sub-site soil samples within the agricultural field under wheat-cotton rotation was found to vary between 16.51 to 237.65 mg kg^{-1} with a CV value of 55.83% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the MBC was found to vary from 47.28 to 232.54 mg kg^{-1} with a CV value of 31.02%. However, among the selected three agricultural fields, the highest CV value (63.69%) was observed within the agricultural field under wheat-rice crop rotation with the MBC values ranging from 20.03 to 465.10 mg kg^{-1}. The statistical analysis through analysis of variance (ANOVA) indicated that the mean (153.55 mg kg^{-1}) of the MBC within the agricultural field under wheat-rice crop rotation was significantly higher as compared to the mean values for the fields under wheat-cotton (110.95 mg kg^{-1}) and wheats-maize (120.22 mg kg^{-1}) crop rotations which were statistically at par with each other.

The microbial biomass nitrogen (MBN) of the sub-site soil samples within the agricultural field under wheat-cotton rotation was found to vary between 1.90 to 24.10 mg kg^{-1} with a CV value of 54.09% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the MBN was found to vary from 4.97 to 20.70 mg kg^{-1} with a CV value of 28.02%. However, among the selected three agricultural fields, the highest CV value (64.43%) was observed within the agricultural field under wheat-rice crop rotation with the MBC values ranging from 1.90 to 42.70 mg kg^{-1}. The statistical analysis through analysis of variance (ANOVA) indicated that the means of the MBN values of the sub-site soil samples within the agricultural fields under wheat-cotton (11.69 mg kg^{-1}), wheats-maize (11.77 mg kg^{-1}) and wheat-rice (13.96 mg kg^{-1}) crop rotations were statistically at par with each other.

The number of culturable heterotrophic bacteria in the sub-site soil samples within the agricultural field under wheat-cotton rotation was found to vary between 1.93×10^7 to 9.57×10^7 cfu g^{-1} with a CV value of 31.80% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the number of culturable heterotrophic bacteria was found to vary from 2.70×10^7 to 1.03×10^8 cfu g^{-1} with a CV value of 28.38%. Similarly, a CV value of 34.41% was observed for the agricultural field under wheat-rice crop rotation with the number of culturable heterotrophic bacteria ranging from 2.34×10^7 to 8.85×10^7 cfu g^{-1}. The statistical analysis through analysis of variance (ANOVA) indicated that the mean (6.70×10^7 cfu g^{-1}) of the number of culturable heterotrophic bacteria within the agricultural field under wheat-maize crop rotation was significantly higher as compared to the mean values of number of culturable heterotrophic bacteria for the fields under wheat-cotton (5.92×10^7 cfu g^{-1}) and wheats-rice (5.11×10^7 cfu g^{-1}) crop rotations. The mean value of the number of culturable heterotrophic bacteria of the soil samples from the field under wheat-cotton rotation (5.92×10^7 cfu g^{-1}) was also significantly higher as compared to the mean number of culturable heterotrophic bacteria of the samples from the agricultural field under wheats-rice (5.11×10^7 cfu g^{-1}) crop rotation.

Characterization of Isoproturon Dissipation/Degradation Kinetics Parameters in the Selected Three Agricultural Fields

In this study, 54 sub-site soil samples from agricultural field under wheat-cotton rotation, 50 sub-site soil samples from agricultural field under wheat-maize rotation and 54 sub-site soil samples from agricultural field under wheat-rice rotation were spiked with 15 mg kg^{-1} of isoproturon and the isoproturon dissipation/degradation from the soil samples were monitored over a 90 days incubation period. The isoproturon dissipation patterns in 54 sub-site soil samples from agricultural field under wheat-cotton rotation, 50 sub-site soil samples from agricultural field under wheat-maize rotation and 54 sub-site soil samples from agricultural field under wheat-rice rotation have been presented in Fig. 1. Interestingly, a good value of isoproturon dissipation was observed in most of the soil samples from the selected three agricultural fields.

The results of isoproturon dissipation over the incubation period (90 days) were modelled using Gompertz model with modifications (Zwietering *et al.*, 1990) and kinetic parameters including maximum isoproturon dissipation [A (%)], the maximum rate of isoproturon dissipation [μm (%.day^{-1})] and the lag period [λ (days)] for sub-site soil samples from the selected three agricultural fields were calculated. Descriptive statistics including minimum/maximum values, mean/median, SD and CV for the isoproturon dissipation kinetics parameters [A (%), μm (%.day^{-1}) and λ (days)] of the 54 sub-site soil samples from the agricultural field under wheat-cotton rotation, 50 sub-site soil samples from the agricultural field under wheat-maize rotation and 54 sub-site soil samples from the agricultural field under wheat-rice rotation have been presented in Table 2. The values of all the biological parameters were found to be considerably different not only from field to field but also at different points within each field.

The maximum isoproturon dissipation (A) of the sub-site soil samples within the agricultural field under wheat-cotton rotation was found to vary between 13.73 to 73.76% with a CV value of 25.32% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the maximum isoproturon dissipation was found to vary from 23.96 to 79.88% with a CV value of 18.85%. Similarly, a CV value of 24.90% was observed within the agricultural field under wheat-rice crop rotation with the maximum isoproturon dissipation values ranging from 13.48 to 71.11%. The analysis of variance indicated that the mean (58.79%) of the maximum isoproturon dissipation within the agricultural field under wheat-maize crop rotation was significantly higher as compared to the mean values for the fields under wheat-cotton (52.45%) and wheats-rice (49.75%) crop rotations which were statistically at par with each other (Fig. 2).

The maximum rate of isoproturon dissipation (µm) of the sub-site soil samples within the agricultural field under wheat-cotton rotation was found to vary between 0.50 to 2.32%.day^{-1} with a CV value of 31.16% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the μm was found to vary from 0.40 to 2.98%.day^{-1} with a CV value of 34.10%. Similarly, a CV value of 29.13% was observed within the agricultural field under wheat-rice crop rotation with the µm values ranging from 0.29 to 1.62%.day^{-1}. The statistical analysis through analysis of variance (ANOVA) indicated that the mean (1.15%.day^{-1}) of the µm within the agricultural field under wheat-rice crop rotation was significantly lower as compared to the mean values for the fields under wheat-cotton

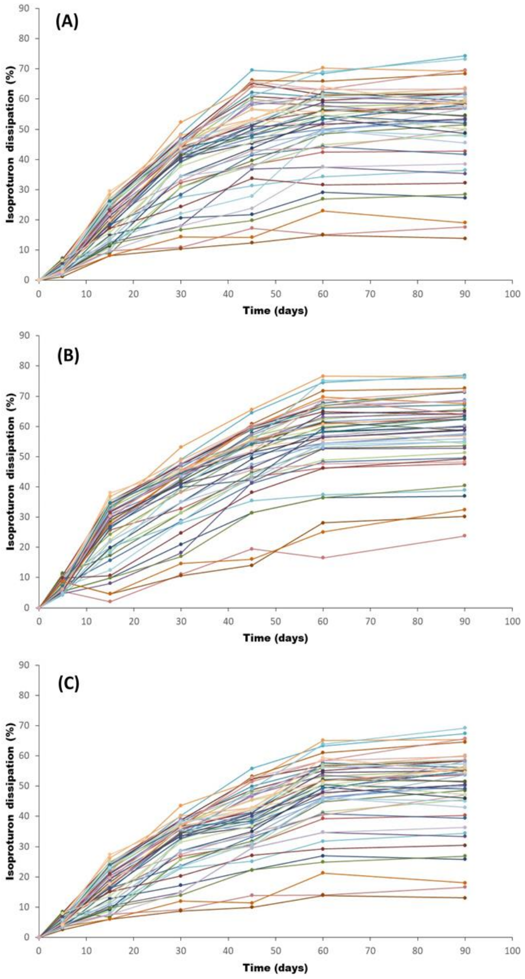

Fig. 1: Dissipation of the spiked isoproturon from the soil samples under wheat-cotton (**A**), wheat-maize (**B**) and wheat-rice (**C**) crop rotation over an incubation period of 90 days at 25°C

(1.64%.day^{-1}) and wheats-maize (1.62%.day^{-1}) crop rotations which were statistically at par with each other (Fig. 2).

The lag period (λ) during isoproturon dissipation in the sub-site soil samples within the agricultural field under wheat-cotton rotation was found to vary between 2.47 to 14.37 days with a CV value of 30.32% (Table 2). At different points within the agricultural field under wheat-maize crop rotation, the lag period was found to vary from 1.30 to 17.17 days with a CV value of 50.58%. Similarly, a CV value of 46.33% was observed within the agricultural field under wheat-rice crop rotation with the lag periods ranging from 0.10 to 14.69 days. The statistical analysis through analysis of variance indicated that the mean (7.01 days) of the lag periods within the agricultural field under wheat-cotton crop rotation was significantly higher as compared to the mean values for the fields under wheat-rice (5.85 days) and wheats-maize (5.34 days) crop rotations which were statistically at par with each other (Fig. 2).

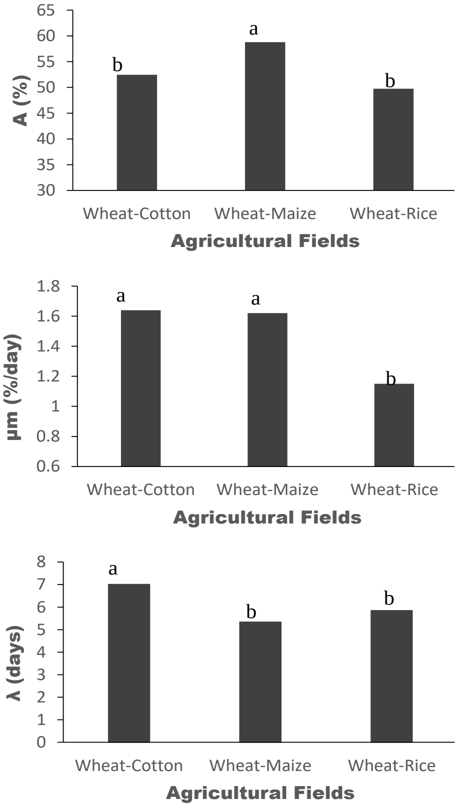

Fig. 2: Average of the Gompertz Model parameters (A, *µ*m & λ) of the soil samples from three agricultural fields (54 from wheat-cotton, 50 from wheat-maize, 54 from wheat-rice field): A represents the maximum isoproturon dissipation (%), *µ*m represents the maximum rate of isoproturon dissipation (% day^{-1}) and **λ** represents the lag period (days)

Linking the Isoproturon Dissipation Kinetics Parameters with Physicochemical and Biological Parameters in the Selected Three Agricultural Fields

The correlation of the modelled isoproturon dissipation kinetics parameters with the physicochemical and biological properties of the soils under the selected three agricultural fields was estimated by calculating the Pearson's coefficient of correlation. The values of the Pearson's coefficient of correlation between the modelled parameters (A, *µ*m, λ) and the physicochemical/biological parameters (pH, EC, OM, DOC, TOC, CEC, MBC, MBN, cfu) of the 54 sub-site soil samples from agricultural field under wheat-cotton rotation, 50 sub-site soil samples from agricultural field under wheat-maize rotation and 54 sub-site soil samples from agricultural field under wheat-rice rotation have been presented in Table 3. The data indicate that the modelled parameters were correlated with different physicochemical and biological parameters but with varying extents and directions. On the basis of the correlation matrices calculated for all the parameters of the sub-site soil samples within the agricultural fields under wheat-cotton, wheat-maize and wheat-rice (Fig.

3) crop rotations, it can be found that the maximum isoproturon dissipation (A) and the maximum rate of isoproturon dissipation (μm) were significantly positively correlated with the TOC, OM content and the number of culturable heterotrophic microorganisms (cfu g^{-1} soil). According to these matrices, the maximum isoproturon dissipation (A) and the maximum rate of isoproturon dissipation (μm) were significantly negatively correlated with the pH in all three agricultural fields. As per data obtained from this study, the lag period (λ) was not found to be correlated with any of the physicochemical or biological parameters except in the soil samples from the agricultural field under wheat-maize crop rotation in which the lag period was significantly negatively correlated with the culturable heterotrophic microorganisms (cfu g^{-1} soil) and significantly positively correlated with the pH (Fig. 3). The rest of the parameters were not significantly correlating with any of the three modelled isoproturon dissipation kinetics parameters.

pH is an important factor which can affect the dissipation and biodegradation of different organic compounds including the pesticides in soils. The maximum isoproturon dissipation (A) was found to be significantly negatively correlated with the pH of the soil samples from the agricultural fields under wheat-cotton ($r = -0.5334$, $R^2 = 0.2845$), wheat-maize ($r = -0.5241$, $R^2 = 0.2746$) and wheat-rice ($r = -0.6115$, $R^2 = 0.3739$) crop rotations. Like the maximum isoproturon dissipation (A), the maximum rate of isoproturon dissipation (μm) was also found to be negatively correlated with the pH of the soil samples from the agricultural fields under wheat-cotton ($r = -0.6842$, $R^2 = 0.4681$), wheat-maize ($r = -0.7165$, $R^2 = 0.5134$) and wheat-rice ($r = -0.6862$, $R^2 = 0.4709$) crop rotations. However, the lag period (λ) was not found to be significantly correlated with the pH of the soil samples from the agricultural fields under wheat-cotton (Fig. 3A) and wheat-rice (Fig. 3C) crop rotations. Nevertheless, the lag period was significantly positively correlated ($r = 0.3817$, $R^2 = 0.1457$) with the pH of the soil samples collected from the agricultural fields under wheat-maize crop rotation (Fig. 3B).

The maximum isoproturon dissipation (A) was found to be significantly positively correlated with the TOC of the soil samples from the agricultural fields under wheat-cotton ($r = 0.4531$, $R^2 = 0.2053$), wheat-maize ($r = 0.4184$, $R^2 = 0.1751$) and wheat-rice ($r = 0.4624$, $R^2 = 0.2138$) crop rotations. Like the maximum isoproturon dissipation (A), the maximum rate of isoproturon dissipation (μm) was also found to significantly positively correlated with the TOC of he soil samples from the agricultural fields under wheat-cotton ($r = 0.4443$, $R^2 = 0.1974$), wheat-maize ($r = 0.6401$, $R^2 = 0.4096$) and wheat-rice ($r = 0.5179$, $R^2 = 0.2682$) crop rotations. However, the lag period (λ) was not found to be significantly correlated with the TOC of the soil samples from the agricultural fields under wheat-cotton, wheat-maize and wheat-rice (Fig. 3) crop rotations having the coefficient of correlation (r) values of - 0.0515, - 0.2599 and - 0.2120 (Table 3), respectively.

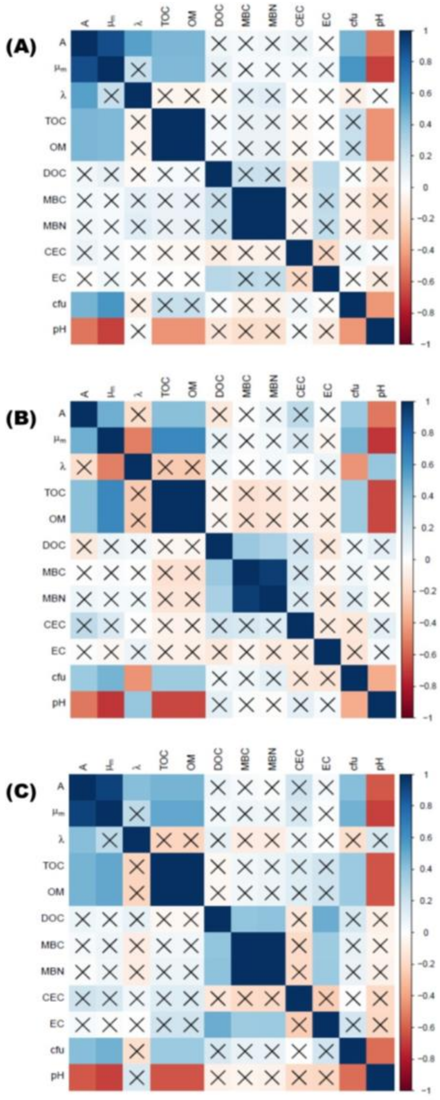

Fig. 3: Correlation matrix between the physicochemical, biological and isoproturon dissipation kinetics parameters measured from 54 sub-site soil samples collected from the agricultural field under wheat-cotton crop rotation (**A**), 50 sub-site soil samples collected from the agricultural field under wheat-maize crop rotation (**B**) and 54 sub-site soil samples collected from the agricultural field under wheat-rice crop rotation (**C**). The color scale on the right side of the figure shows the intensity as well as the direction of the correlation and the crossed (×) boxes indicate that the correlation is not significant

The maximum isoproturon dissipation (A) was found to significantly positively correlated with the number of the culturable heterotrophic microorganisms of the soil samples from the agricultural fields under wheat-cotton ($r = 0.4663$, $R^2 = 0.2174$), wheat-maize ($r = 0.3616$, $R^2 = 0.1308$) and wheat-rice ($r = 0.4235$, $R^2 = 0.1794$) crop rotations. Like the maximum isoproturon dissipation (A), the maximum rate of isoproturon dissipation (μm) was also found to be significantly positively correlated with the number of the

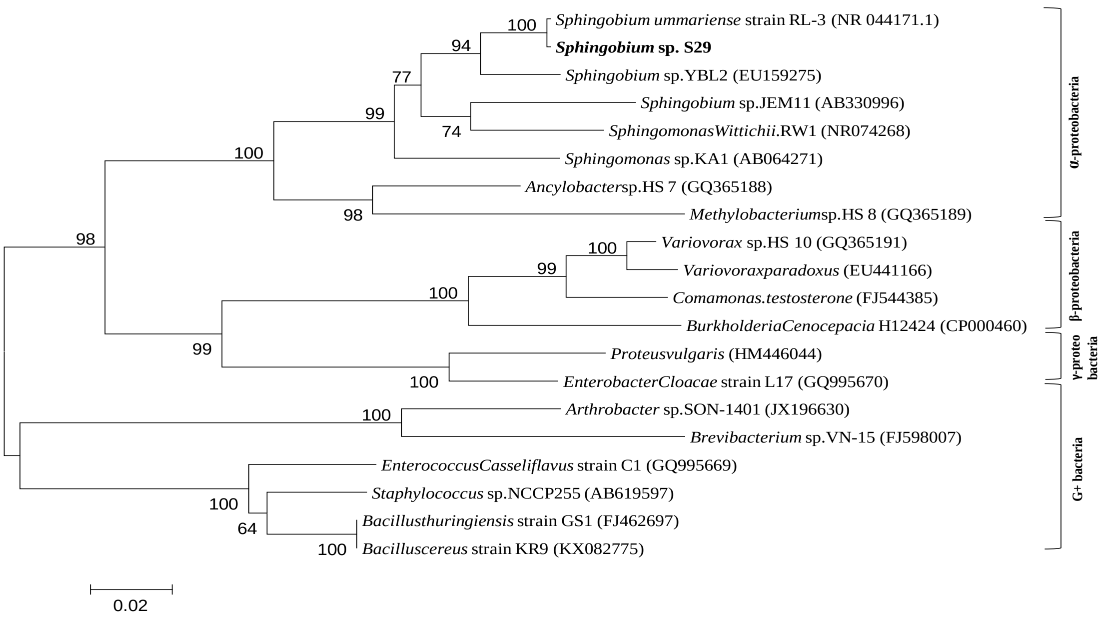

Fig. 4: Phylogenetic tree for comparison of *Sphingobium* spp. S29 with the known bacterial strains in the GenBank

culturable heterotrophic microorganisms of the soil samples from the agricultural fields under wheat-cotton (r = 0.5807, R^2 = 0.3373), wheat-maize (r = 0.4616, R^2 = 0.2131) and wheat-rice (r = 0.4723, R^2 = 0.2231) crop rotations. However, the lag period (λ) was not found to be significantly correlated with the number of the culturable heterotrophic microorganisms of the soil samples from the agricultural fields under wheat-cotton (Fig. 3A) and wheat-rice (Fig. 3C) crop rotations. Nevertheless, the lag period was significantly negatively correlated (r = - 0.4479, R^2 = 0.2006) with the number of the culturable heterotrophic microorganisms of the soil samples collected from the agricultural fields under wheat-maize crop rotation (Fig. 3B).

Isolation and Characterization of the Strain S29

While isolating the bacterial strain having the potential for degradation of isoproturon, it was observed that several colonies appeared on MS agar media plates. Out of these colonies, 24 colonies with relatively varying morphological characteristics were tested for their potential to degrade isoproturon in MS medium. However, one colony type was found dominant on the plates. The same colony type was found to degrade isoproturon in the MS broth medium. This colony type was round creamy in color with smooth edges. The phylogenetic analysis of the isolate S29 indicated that this strain was grouped with the bacterial strains belonging to genus *Sphingobium* (Fig. 4). On the basis of this phylogenetic analysis as well as its identity with the *Sphingobium* strains through BlastN analysis, this strain was designated as *Sphingobium* spp. S29.

The strain S29 had almost completely (>90%) degraded the initially added isoproturon only within 46 h. However, it could not completely degrade any of the other phenylurea herbicides under the set conditions (Fig. 5A). The degradation of linuron and monolinuron by the strain S29 was almost negligible. Nevertheless, it had degraded about 66.2% and 38.1% of the initially added diuron and chlorotoluron over the 58 h incubation period, respectively.

While studying the degradation of known isoproturon metabolies, the strain S29 was found to degrade all the three known metabolies of isoproturon *viz.* monodemethyl-isoproturon, didemethyl-isoproturon and 4-isoprpyl aniline (Fig. 5B). This strain had almost completely (>90%) degraded the initially added isoproturon, monodemethyl-isoproturon, didemethyl-isoproturon and 4-isoprpyl aniline over the incubation periods of 48 h, 48 h, 72 h and 54 h, respectively.

Discussion

This research work was carried out to estimate the pesticides dissipation variability not only in the agricultural fields under different types of crop rotations but also at different points within the agricultural fields using isoproturon as a model pesticide. For this study, 54 sub-site soil samples were collected from an agricultural field under wheat-cotton rotation, 50 sub-site soil samples from an agricultural field under wheat-maize crop rotation and 54 sub-site soil samples from an agricultural field under wheat-rice crop rotation (Fig. 1). These agricultural fields were variable not only in their crop rotations but also in their textures (Table 1). The fields under wheat-cotton, wheat-maize and wheat-rice crop rotations were having the sandy loam, loam and clay loam soil textures, respectively. All the collected samples from the

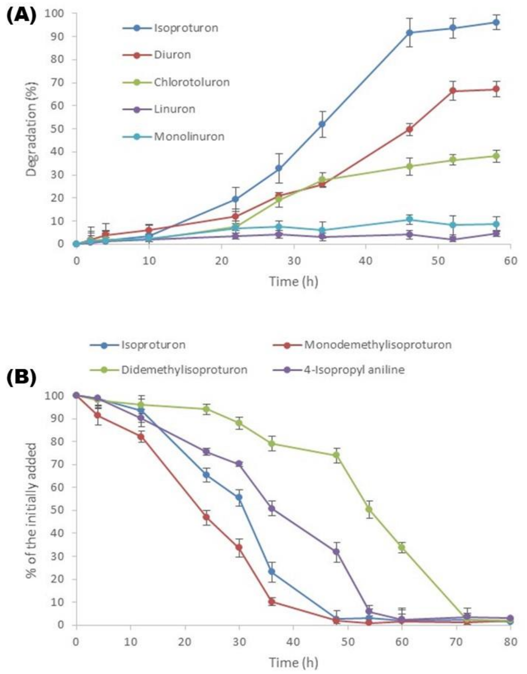

Fig. 5: Biodegradation of different phenylurea herbiicides (**A**) and different known metabolites of isoproturon (**B**) by *Sphingobium* spp. S29 in MS medium

selected three agricultural fields were analyzed for different physicochemical and biological parameters (Table 2). The samples were also evaluated for their potential in terms of isoproturon dissipation after spiking with isoproturon (15 mg kg^{-1} of soil). The obtained isoproturon dissipation data modelled using a modified Gompertz Model (Zwietering *et al*., 1990) and the modelled parameters including the maximum isoproturon dissipation [A (%), the maximum rate of isoproturon dissipation [μm (%.day^{-1})] and lag period [λ (days)] were obtained.

The results of our study indicated that the maximum isoproturon dissipation (A) ranging from 13.48 to 79.88% and the maximum rate of isoproturon dissipation (μm) ranging from 0.40 to 2.98%.day^{-1} was recorded in all the samples collected from the selected three agricultural fields (Table 2). This finding indicates that the soils in all three selected fields were adapted for isoproturon dissipation and degradation as a result of repeated application of this herbicide for more than ten previous years. This finding is in accordance with studies in which such an adaptation for isoproturon degradation has been reported in response to its repeated application (Walker *et al*., 2001; El-Sebai *et al*., 2007; Hussain *et al*., 2013). Not only for isoproturon but such adaptability has also been reported for degradation and dissipation of a few other pesticides including diuron, chlorotoluron and diflufenican (Walker *et al*., 2002; Bending *et al*., 2006; Hussain *et al*., 2015). A wide range of variation in the modelled parameters 'A' and 'μm' of the sub-site soil samples is also in accordance with a number of previous studies who reported for a spatial variability in isoproturon mineralization kinetics at different points within the same agricultural field (El-Sebai *et al*., 2007; Hussain *et al*., 2013). For example, Hussain *et al*. (2013) reported for a temporal as well as spatial variability in isoproturon mineralization within an agricultural field located in Epoisses, France over a three years crop rotation from 2008 to 2010. Interestingly, the highest mean values of 'A' was observed in the soil samples collected from the agricultural field under wheat-maize crop rotation which was significantly higher than the mean values obtained from the fields under wheat-cotton and wheat-rice crop rotations. However, this highest value of 'A' in wheat-maize field was followed by that of in the field under wheat-cotton crop rotation. The mean values of 'μm' in the soil samples under wheat-maize and wheat-cotton crop rotations were at par with each other but significantly higher than that of in the soil samples under wheat-rice crop rotation. This indicates that the potential for isoproturon dissipation was highest in the soils under wheat-maize crop rotation followed by that of in the soils under wheat-cotton crop rotation. This variability in isoproturon dissipation in the soils under varying crop rotations might be due to

Table 3: Pearson's coefficient of correlation (r) between the modelled parameters [maximum isoproturon dissipation (A), maximum rate of isoproturon dissipation (μm) and lag period (λ)] and the physicochemical parameters [pH, electrical conductivity (EC), organic matter (OM) content, dissolved organic carbon (DOC), total organic carbon (TOC), cation exchange capacity (CEC), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and colony forming units (cfu)] of the soils from the selected three agricultural fields with varying crop rotations

Parameter *vs*. Parameter			Coefficient of correlation (r)		
			Wheat-Cotton	Wheat-Maize	Wheat Rice
A	*vs.*	pH	-0.5334	-0.7165	-0.6115
		EC	-0.0085	0.0142	0.0379
		OM	0.4531	0.4184	0.4624
		DOC	0.0534	-0.1115	0.0629
		TOC	0.4531	0.4184	0.4624
		CEC	0.0761	0.2502	0.2016
		MBC	0.0423	-0.0009	0.0303
		MBN	0.0511	0.0609	0.0155
		cfu	0.4663	0.3616	0.4235
μm	*vs.*	pH	-0.6842	-0.5241	-0.6862
		EC	0.0448	-0.0366	0.0128
		OM	0.4443	0.6401	0.5179
		DOC	0.0982	0.0703	0.0473
		TOC	0.4443	0.6401	0.5179
		CEC	0.0311	0.1598	0.1617
		MBC	0.0327	0.0290	0.0653
		MBN	0.0370	0.0488	0.0592
		cfu	0.5807	0.4616	0.4723
λ	*vs.*	pH	0.0127	0.3817	0.1604
		EC	-0.0081	0.0797	0.0049
		OM	-0.0515	-0.2599	-0.2120
		DOC	-0.0289	0.0518	0.0969
		TOC	-0.0515	-0.2599	-0.2120
		CEC	-0.0079	0.0088	0.0533
		MBC	0.1023	-0.0019	-0.0963
		MBN	0.1214	0.0460	-0.0927
		cfu	-0.0787	-0.4479	-0.1673

varying physicochemical and biological parameters in three fields as already reported for several other agricultural fields having varying physicochemical and biological properties (Walker *et al*., 2002; El-Sebai *et al*., 2007; Hussain *et al*., 2013). In this study, the soils under wheat-maize crop rotation showing the highest mean potential for isoproturon dissipation were found to have the highest mean values of DOC, TOC, OM content and the number cultural heterotrophic microorganisms, and the lowest values of pH which might be driving force for this highest potential.

In order to ascertain the key parameters playing their role in modifying the isoproturon dissipation activity in the soil samples from the selected three agricultural fields, the values of correlation were calculated between the modelled isoproturon dissipation parameters (A, μm & λ) and the physicochemical as well as the biological parameters for the soil samples. In this evaluation, pH, TOC, OM content and culturable heterotrophic bacteria were identified as the key parameters which were affecting the isoproturon dissipation kinetics in different soils samples. In this study, pH was found to significantly negatively correlated with 'A' as well as 'μm' in the soils from all three selected agricultural fields. These soils were having the pH in alkaline range of more than 8.25. This finding indicates that a decrease in pH from these values could result into an increase in the isoproturon dissipation in the soils. This finding is in accordance with a number of previous studies which have already reported the pH as one of the key parameters playing the role in regulating the degradation/dissipation of the pesticides including isoproturon in soil media as well as the aqueous media (Bending *et al*., 2001; Walker *et al*., 2001; El-Sebai *et al*., 2005, 2007; Hussain *et al*., 2009, 2013, 2017; Li *et al*., 2017). For example, while studying the isoproturon mineralization in a French agricultural field of Le Souich, El-Sebai *et al*. (2007) found that the in-field spatial distribution of isoproturon mineralization was correlated with pH at different points within that agricultural field. Similar results were reported by Hussain *et al*. (2013) while studying the isoproturon mineralization in a French agricultural field of Epoisses. In addition to the studies in agricultural soils, the impact of pH on isoproturon degradation has also been reported in a number of studies focused on pure cultures of bacteria in aqueous media and a pH ranging from neutral to slightly alkaline was observed for an optimum activity (Bending *et al*., 2001; Sorensen *et al*., 2003; Hussain *et al*., 2009). All these previous studies and the findings of the present study indicate that pH is one of the key factors for regulating the isoproturon dissipation in the soils. In addition to pH, the modelled isoproturon dissipation kinetics parameters were found to be significantly positively correlated with the TOC and OM contents of the soils samples collected from the selected three agricultural soils. Like pH, organic matter is also an important parameter which plays a significant positive role in regulating the isoproturon dissipation activity in varying agricultural soils analyzed in this study. A few previous studies have also reported the important role of the organic matter content in regulating the biodegradation of different pesticides including isoproturon in various agricultural soils (Saison *et al*., 2006; Briceno *et al*., 2007; Hussain *et al*., 2013, 2015, 2017). The presence of organic matter increases the pesticides dissipation from the agricultural soils by proliferating the microbial population as well as the activity. In the present study, the number of culturable heterotrophic microorganisms was also found to be significantly positively correlated with the isoproturon dissipation in the soils from different agricultural fields. This finding indicates for a possible role of the microbial communities in the dissipation of isoproturon from these soils. Although the cultural heterotrophic microorganisms is an important factor controlling the isoproturon dissipation in the soils under this study, however, some of the previous studies indicate that this factor does not play any significant role in isoproturon dissipation (El-Sebai *et al*., 2007; Hussain *et al*., 2013). Nevertheless, the abundance of culturable heterotrophic microorganisms is considered as a useful factor in controlling the isoproturon dissipation in soils in the present study.

In this study, a strain S29 was isolated for degradation of isoproturon. Blastn and phylogenetic analyses of this strain indicated that this strain belonged to genus *Sphingobium*. Hence, it was designated as *Sphingobium* spp. S29. Previously, a few isoproturon degrading strains have been isolated from geographically different regions (Sorensen *et al*., 2003; El-Sebai *et al*., 2004; Sun *et al*., 2009; Dwivedi *et al*., 2011; Hussain *et al*., 2011). However, it is interesting to note that the most of these a few isoproturon degrading bacterial strains are *Sphingomonads* belonging to genera *Sphingobium* and *Sphingomonas* (Sorensen *et al*., 2003; Sun *et al*., 2009; Dwivedi *et al*., 2011; Hussain *et al*., 2011; Yan *et al*., 2016; Hussain *et al*., 2017). This indicates the existence of a good potential of isoproturon degradation in the bacteria belonging to these two genera. Hence, the present strain S29 will serve as a new potential addition in the isoproturon degrading bacterial strains belonging to these two genera. This strain also had the potential to degrade the known metabolites of isoproturon. Such potential for degradation of the commonly known metabolites of isoproturon (monodemethyl-isoproturon, didemethyl-isoproturon and 4-isoprpyl aniline) has also been reported in *Sphingomonas* sp. SH (Hussain *et al*., 2011), *Sphingomonas* spp. SRS2 (Sorensen *et al*., 2003) and *Sphnigobium* spp. YBL2 (Sun *et al*., 2009). Interestingly, all of these strains also have the potential for mineralization of isoproturon. This indicates that the strain S29 might also the potential for mineralization of isoproturon. However, there is need to test this potential. It is also noteworthy, that this strain could also considerable degrade two other tested phenylurea herbicides *viz.* diuron and chlorotoluron. This shows for a broad spectrum capabilities of the strain S29 to degrade different phenylurea herbicides. Previously, Sorensen *et al*. (2013) found that the strain SRS2 had the potential to degrade different phenylurea herbicides. However, *Methylopila* spp. TES (El-Sebai *et al*., 2004) and *Sphingomonas* spp. SH (Hussain *et al*., 2011) had the very specific potential only for degradation of isoproturon.

Conclusion

Selected three soils repeatedly treated with isoproturon over a period of more than ten years are adapted for isoproturon dissipation and that the isoproturon dissipation is variable not only in different types of agricultural soils under varying types of crop rotations but also at different points within the same agricultural field. Isoproturon dissipation variability within same field was positively correlated to the organic matter content, total organic carbon as well as the abundance of culturable heterotrophic microorganisms and negatively correlated with the pH. It is also concluded that the strain *Sphingobium* spp. S29 has a good potential for degradation of different phenylurea herbicides and the metabolites of isoproturon. However, there is need to further characterize the capabilities of the strain S29.

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