ORIGINAL RESEARCH

Biodegradation of topramezone by a *Trichoderma* Isolate in soil

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Received: 22 April 2019

Accepted for publication: 20 June 2019

Published: August 2019

Abstract

Topramezone, a pyrazolone compound, has been introduced in many countries as a post-emergence herbicide. It inhibits 4-hydroxyphenylpyruvate dioxygenase (HPPD), a key enzyme in carotenoid pigment biosysnthesis, in susceptible plants, and can effectively control annual grasses and broadleaf weeds in maize (*Zea mays* L.), sweet corn (*Zea mays* convar. *Saccharata* var. *rugosa*) and popcorn (*Zea mays* var. *everta*). Topramezone is a highly persistent herbicide, which has high mobility in soil, posing a risk of leaching to ground and surface water. Despite its increasing use, not much is known about topramezone degradation and the potential impact of its persistence in the agricultural environment.

We investigated the interaction between the herbicide and soil microorganisms in topramezone-treated soil, in order to test its bio-remediation potential particularly by soil fungi, and to elucidate the possible degradation pathways. One microbial strain, capable of transforming topramezone, was isolated from soils treated with the herbicide and identified as a species of *Trichoderma*, a well-known, common soil organism. The isolate survived in the minimal broth, incorporated with topramezone, at a concentration of 1000 mg/L of the medium. In sterilized soil, spiked with the herbicide, the *Trichoderma* isolate degraded 85% of the applied topramezone within 30 days of incubation, which is much faster than the reported, standard half-life of the herbicide (about 120 days). Based on the eight breakdown products (I to VIII), which were identified by liquid chromatography-mass spectroscopy (LC-MS) analyses, we propose that the herbicide was degraded by the fungus through various biochemical reactions, viz. demethylation, desulfonylation followed by hydroxylation of the herbicides, alkyl hydroxylation, hydrolysis of the carbonyl group of ketone, methoxylation, and hetero ring hydroxylation. Our results add to previous research that *Trichoderma* species and its strains are capable of degrading some pesticides, including herbicides in soil. The degradation products identified strongly imply the presence of a substrate recognition mechanism and a corresponding metabolic response system in the *Trichoderma* isolate, which can effectively degrade topramezone in the agricultural soil.

Keywords: Biodegradation, Bioremediation, Herbicide, Topramezone, *Trichoderma* sp.

Introduction

Topramezone, a pyrazolone herbicide, has been introduced for crop protection in several countries, recently (Porter et al., 2005; Anonymous, 2006; Soltani et al., 2007). It is a selective, post-emergent herbicide, which controls broad-leaf weeds and several grasses in maize (*Zea mays* L.), sweet corn (*Zea mays* convar. *Saccharata* var. *rugosa*) and popcorn (*Zea mays* var. *everta*), at a low application rate of 25.2-33.6 g a.i./ha (Anonymous, 2006; Anonymous, 2018). The mode of action of topramezone is different from many other common

herbicide groups, such as urea herbicides, sulfonyl ureas and imidazolinones, and carbamate herbicides. It acts by inhibiting the activity of the enzyme: 4-hydroxyphenylpyruvate dioxygenase (4-HPPD, EC 1.13.11.27), disrupting the biosynthesis of carotenoid pigments. It has been considered a strong choice for herbicide resistance management in some situations (Anonymous, 2006) because weeds of corn fields have developed resistance against triazines and ALS inhibiting herbicides in many countries, viz. Argentina, Austria, Belgium, Brazil, Bulagaria, Canada, Chile, China, Czech Republic, France, Germany, Italy, Mexico, New Zealand, Spain, Switzerland, and United States (Heap, 2019). Czech Republic, France, Germany, Italy, Mexico, New Zealand, Spain, Switzerland, and United States (Heap, 2019). In these countries, topramezone formulations have been registered as a post-emergent herbicide for maize, in recent times. The herbicide has also been registered in India, since 2015, for post-emergent weed control in maize.

The use of various topramezone formulations is increasing in maize-growing countries. However, not much is known of the degradation pathways or the impact of the herbicide in the agricultural environment, following applications. Topramezone is highly persistent in different soils. In the USA, in aerobic soil, its half-life has been determined to be more than 125 days (USEPA, 2005). It does not undergo hydrolysis or photolysis in soil and water, readily. The dissipation of this compound is largely dependent on sorption, over time. Moreover, topramezone has high mobility in soils and sediments, presenting a risk of leaching into ground and surface water (USEPA, 2005; Stipičević et al., 2016). There are no reports yet available on the biodegradation of topramezone in soil. Due to its higher chemical or photo-chemical stability in the environment, biodegradation by soil organisms is likely to be the natural way by which the herbicide concentrations may decrease in treated soil. Biodegradation is also considered as one of the most effective methods that might be manipulated to expedite the reduction of the concentrations of this herbicide from agricultural soil.

In previous studies, it has been observed that residues of many pesticides could be degraded by the augmentation of various species of the fungal genus *Trichoderma*, either in artificial media, or in soil. The genus *Trichoderma* is a large group of free-living fungi, commonly inhabiting soil and root ecosystems of plants. Some are well known to be beneficial microorganisms for the growth of crop plants. Some strains of *Trichoderma* control many soil-borne phytopathogenic fungi (Harman et al., 2004; Druzhinia et al., 2011), thereby, assisting crop plants to enhance root growth and development (Contreras-Cornejo et al., 2009), increasing nutrient uptake and inducing crop resistance to abiotic stresses (Yasmeen and Siddiqui, 2017).

Presently, the augmentation of effective *Trichoderma* strains in soil through various commercial formulations is gaining importance, both as a biocontrol agent, and a growth promoting agent. Augmentation of *Trichoderma* populations in soil is also useful for expediting the breakdown of pesticide residues in soil, as the genus and *Trichoderma* strains have long been recognized as effective bio-remediation agents in pesticide-contaminated soil. For instance, in some early studies, Kaufman and Blake (1973) found that the augmentation of *T. viride* in silty clay loam soil and in media resulted in an increased degradation and dehalogenation of a variety of pesticides, including chlorphenamidine, chlorpropham, dicryl, diuron, propanil, propachlor, propham and solan. In 1995, Smith observed the influence of different species of *Trichoderma* isolated from forest soil samples in the presence of persistent organochlorine contaminants. In their studies, two species of *Trichoderma, T. harzianum* and *T. viride*, degraded organochlorine pesticides *in vitro.* Both species of *Trichoderma* were also reported to be capable of degrading a broad range of other xenobiotics, including endosulfan, cyanide, phenanthrene, pyrene, and pentachlorophenol (Cserjesi, 1967; Katayama and Matsumura, 1993; Ravelet et al., 2000; Chavez-Gomez et al., 2003; Ezzi and Lynch, 2005).

Other studies have also shown that *T. viride* could degrade persistent pesticides, such as chlorpyrifos and photodieldrin (Tabet and Lichtenstein, 1976; Mukherjee and Gopal, 1996). More recently, Askar et al. (2007) reported that *T. viride* and *T. harzianum* degraded bromoxynil very efficiently, over 98% within 28 days after incubation in media. In addition, Abd-Alrahman et al. (2013) observed that *T. viride* could also degrade butachlor to the extent of 98% within 15 days. A marine-derived *Trichoderma* sp. (CBMAI 932) was also demonstrated to be capable of utilizing chlorpyrifos as a sole nutrient source by hydrolyzing it in distilled water (Alvarenga et al., 2015). The microbe was able to degrade 72% of the applied chlorpyrifos in media, and reduce the concentration of 3,5,6-trichloro-2-pyridinol, the metabolite formed by the enzymatic hydrolysis of chlorpyrifos. The cleavage of the

sulfonyl urea bridge was suggested to take place through a pH dependent reaction. However, a strain of *Trichoderma* was found effective in cleaving the sulfonyl urea bridge enzymatically (Yadav and Choudury, 2014). The augmentation of *Trichoderma* sp. in soil, fortified with sulfosulfuron, a sulfonyl urea herbicide, led to the hydrolysis of sulfonyl urea bond with the formation of two degradation products, viz. 2-ethylsulfonyl imidazo {1,2-a} pyridine-3-sulfonamide and 2-amino-4,6-dimethoxypyrimidine. Thus, *Trichoderma* species clearly have the capacity to degrade pesticides of different chemical groups.

Given the above, the objectives of our study were to investigate topramezone degradation by *Trichoderma* sp., occurring in herbicide-treated soil, and the possible herbicide degradation pathways. Understanding the process of topramezone degradation in soil is important because it could lead to augmenting the organisms who are capable of causing the degradation of the herbicide, as a possible soil remediation option in the future.

Materials and Methods

Chemicals

An analytical grade sample of topramezone was obtained from the Sigma-Aldrich Corporation. Technical grade topramezone was prepared from the formulation, extracting it in dichloromethane, and further purification by repeated crystallization from chloroform and hexane, to a mass of very fine white powder, with a steady melting point of 221-222^{0}C. The purity of this technical topramezone was found to be 97.2% when compared with the analytical grade samples by HPLC analysis. All organic solvents and water were HPLC grade and were purchased from Merck India Ltd. Formic acid was acquired from Merck (Darmstadt, Germany).

Soil

Black (vertisol) soil was collected from the rhizosphere zones from topramezone-treated maize plots of the Experimental Farm, located at the ICAR-Directorate of Weed Research (DWR), Jabalpur. No residues of topramezone were found in the soil. To determine physico-chemical properties, the soil was gently crushed and passed through a 2-mm-mesh sieve. The physical texture of the sandy loam soil was: clay (<2 mm) 57%, silt (2-20 mm) 18%, sand (20-2000 mm) 25%, and organic carbon 0.96%. The soil was also chemically characterized as follows: pH 7.08, electrical conductivity (EC) 0.48 dS m^{-1}, and cation exchange capacity (CEC) 33.8 Cmol (p+) kg^{-1}.

Isolation of *Trichoderma* sp., a topramezone-degrading fungus

Soil, collected from the maize root rhizosphere, was fortified with topramezone at the rate of 50 mg per kg of soil. It was then incubated for one week at 30 ± 2^{0}C. The fungi that survived and persisted in the incubated soil were isolated on potato dextrose agar (PDA) plates. These fungi were screened further by incubating for an additional seven days in minimal PDA broth, containing a range of concentrations of topramezone, viz. 10, 50, and 100 mg per 100 mL of broth. The isolates that showed the highest capacity for the possible degradation of topramezone were screened further, based on their growth.

An isolate that showed promise was again inoculated on PDA plates and incubated at 30 ± 2^{0}C. After two days of incubation, the colony morphology of the isolate was studied. Finally, the fungus was characterized, based on its colony morphology and microscopy of spore and conidia structures, in the Pathology Laboratory of the ICAR-DWR Institute.

Degradation kinetic study

The rate of degradation of topramezone by the *Trichoderma* sp. isolate was examined in a sterile soil, which was obtained by autoclaving the soil at 121°C for 30 min. A Topramezone solution, in chloroform, was added to samples of sterilized soil at the rate of 10 mg per kg of soil. Samples (100 g) of treated soil were taken in Erlenmeyer flasks in triplicate for each day sampling. The soil of each flask was then inoculated with one mL of *Trichoderma* sp. spore suspension of a standard turbidity (10^6 spores/mL), measured by the spectrophotometric method. Flasks were then incubated in an aerobic condition, maintained at a temperature of 28 ± 2^{0}C. A set of three flasks containing topramezone-treated soil, without the inoculation with the *Trichoderma* isolate, was also kept under similar conditions, as a control. Samples were withdrawn from each treatment in triplicate, after 0, 5, 10, 20 and 30 days of incubation.

Soil samples of different days of incubation were extracted with ethyl acetate. The soil in each flask was mixed with 50 mL of HPLC grade ethyl acetate and 5 mL distilled water and agitated on a

reciprocal shaker for 30 min at 150 rpm, followed by centrifugation at 3000 RPM for 15 minutes. The supernatant liquid was filtered through cellulose filter paper (Whatman Grade 1, 11 µm) to remove soil particles. The solid portion, deposited in the centrifuge tube, was again extracted twice by the same method. The combined, filtered solution was concentrated to 2-3 mL in a rotary vacuum evaporator at 40°C and made up to a suitable volume with the mobile phase for analysis by high-performance liquid chromatography (HPLC). For the rate kinetic study, the extracted samples were constituted in the mobile phase of a known volume and were further cleaned up through nylon-made membrane filters before HPLC analysis.

A Shimadzu (LC 8200AHT) isocratic HPLC system was used for the chromatographic separation and quantification of topramezone. The HPLC was equipped with an isocratic liquid pump and a photo diode array detector (SPD-M10A). A stainless-steel column of 250 mm length and 4.6 mm internal diameter packed with octadecyl silane, chemically bonded to porous silica particles of 5 µm diameter (SunFire C18, Waters Corporation), was used in the analysis of topramezone. The flow rate of the mobile phase (acetonitrile/water 65:35 v/v-orthophosphoric acid 0.1%) was maintained at 0.8 mL min^{-1}, and the detector wavelength was set at 250 nm for the monitoring of elution. Employing the above set of parameters, linearity was observed over a wide range of concentrations from 0.2 to 10 ppm. The developed chromatographic method resolved the peak for topramezone at a retention time of 2.83 min.

Isolation and characterization of degradation products

The incubated broth and soil samples, collected at different intervals, were partitioned with chloroform. The chloroform layers collected were dried over anhydrous sodium sulfate. A mixture of degradation products was obtained by evaporating the chloroform layers under low pressure in a rotary vacuum evaporator. The products were characterized by liquid chromatography-mass spectroscopy (LC-MS) with positive modes and tandem mass spectrometric (MS/MS) technique. For the structural elucidation of degraded products, an API 3200 Qtrap mass spectrometer of AB Sciex connected to Shimadzu Ultra Fast Liquid Chromatographic system was used.

Mass spectrometry analysis was carried out with electrospray ionization (ESI) in the positive mode (5500 eV) for each sample. The ion source temperature was set at 500°C. The nebulizer and heater gases were adjusted at 30 psi and 55 psi, respectively. Each sample was injected by an infusion device at the rate of 10 μLs^{-1}. The mass spectrum of each compound was developed after scanning the run at different collision energies obtained from the scanning of potential differences between two collision cells from 5 to 50 volt.

RESULTS

Characterization of the topramezone degrading microbe

The fungus, isolated from the soil of maize root rhizosphere, was initially characterized as a *Trichoderma* sp., based on its morphological features (**Figure 1**). It survived in the minimal broth with topramezone at 1000 mg/L of media (**Figure 2**).

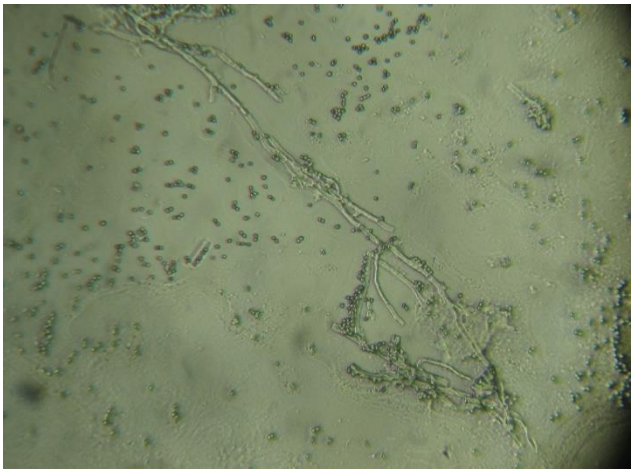

Figure 1. Microscopic characterization: spores, conidiophores and mycelia of the *Trichoderma* isolate

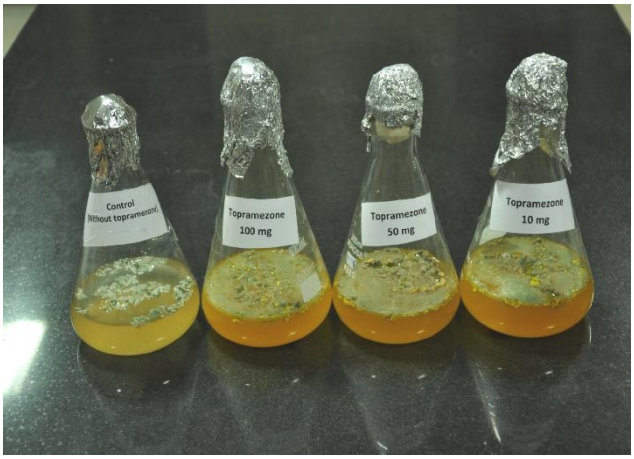

Figure 2. *Trichoderma* isolate growing at different concentrations of topramezone in minimal media

Colonies of the isolate were characteristically fast growing at the temperature of 25-30°C. During the growth period of the filamentous fungus, the colour of the colonies was initially white, which gradually changed to dull white or light green to yellowish green. The mycelia of the isolate were non-septate with a foot-cell. Conidiophores developed erectly inside the branches of hyphae and ended in a terminal, enlarged, ellipsoidal-to-spherical swelling.

The *Trichoderma* isolate is currently being characterized by molecular techniques; i.e. 16S rRNA analysis at the ICAR-Indian Institute of Horticulture Research (ICAR-IIHR), in Bangalore. We believe that the molecular analysis would aid in establishing its identity further in the near future.

The rate of biodegradation of topramezone in soil

The rate of degradation of topramezone in sterilized soil, incubated with the *Trichoderma* isolate, was determined by HPLC analysis of samples collected after different duration times of incubation. The isolate degraded 50% of the applied topramezone within 10 days of incubation and 85% of the herbicide disappeared within 30 days. Initially, the rate of degradation of the herbicide was high, before 10 days, after which the rate declined (**Figure 3**).

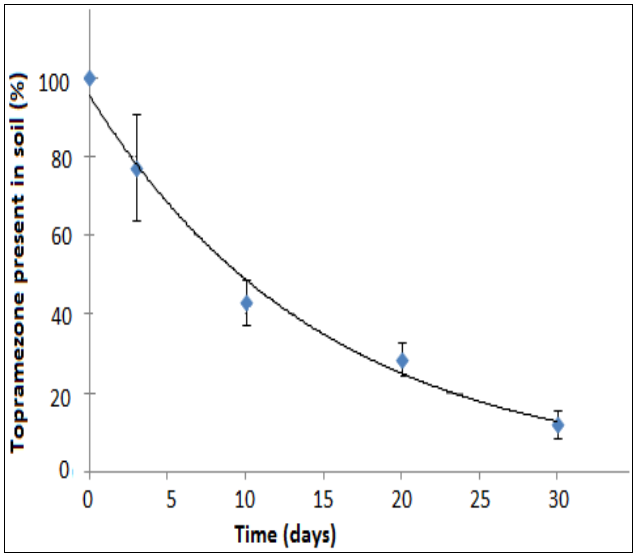

Figure3. Progressive degradation of topramezone in soil incubated with the *Trichoderma* isolate

Metabolism of topramezone by *Trichoderma* sp.

Fungi are known to degrade organic molecules using their own intracellular or extracellular enzymes, including hydrolytic enzymes, peroxidases, oxygenases, etc. (Van Eerd et al., 2003; Ortiz-Hernández et al., 2011). Although, we did not isolate any enzymes in our studies, it is possible to speculate that the isolated strain of *Trichoderma* sp. degraded topramezone both in the media and in soil by releasing intracellular or extracellular enzymes, which acted upon the herbicide, converting it into simpler forms of organic molecules.

Microorganisms are known to utilize these enzyme-driven degradation reactions to derive energy for their growth and maintenance or to detoxify the pesticide (Becker and Seagren, 2010).

In the present study, eight key metabolites produced during the degradation of topramezone by the *Trichoderma* isolate were confirmed by mass spectra and by comparisons with related literature. In media, topramezone was degraded to five major products: (I), (II), (III), (IV), and (VIII), the mass fragmentation pattern of which are given in **Figure 4**. Their chemical structures are given in **Figure 5**. The metabolites we identified were as follows:

I: [3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesylphenyl] (5-hydroxy-1-methyl-pyrazol-4-yl) methanone; II: [3-(4,5-dihydro-1,2-oxazol-3-yl)-4-hydroxy-*o*-tolyl] (5-hydroxy-1-methyl-pyrazol-4-yl) methanone; III: [3-(4,5-dihydro-1,2-oxazol-3-yl)-2-hydroxymethyl-4-hydroxyphenyl] (5-hydroxy-1-methyl-pyrazol-4-yl) methanone; IV: [3-(4,5-dihydro-1,2-oxazol-3-yl)-4-hydroxyphenyl] (5-hydroxy-1-methyl-pyrazol-4-yl) methanone; V: 2-(4,5-dihydro-1,2-isoxazol-3-yl)-4-hydroxytoluene; VI: 1-hydroxymethyl-5-hydroxy-4-pyrazolecarboxylic acid, VII: 1-methoxy-4-hydroxy-4,5-dihydroisoxazole, and VIII: 1-methoxy-4,5-dihydro-isoxazole].

Metabolites II, III and VIII were also found in the topramezone-fortified-soil incubated with the *Trichoderma* isolate. Other metabolites of topramezone isolated from the incubated soil were: (V), (VI), and (VII) (shown in **Figure 4** and **Figure 5**).

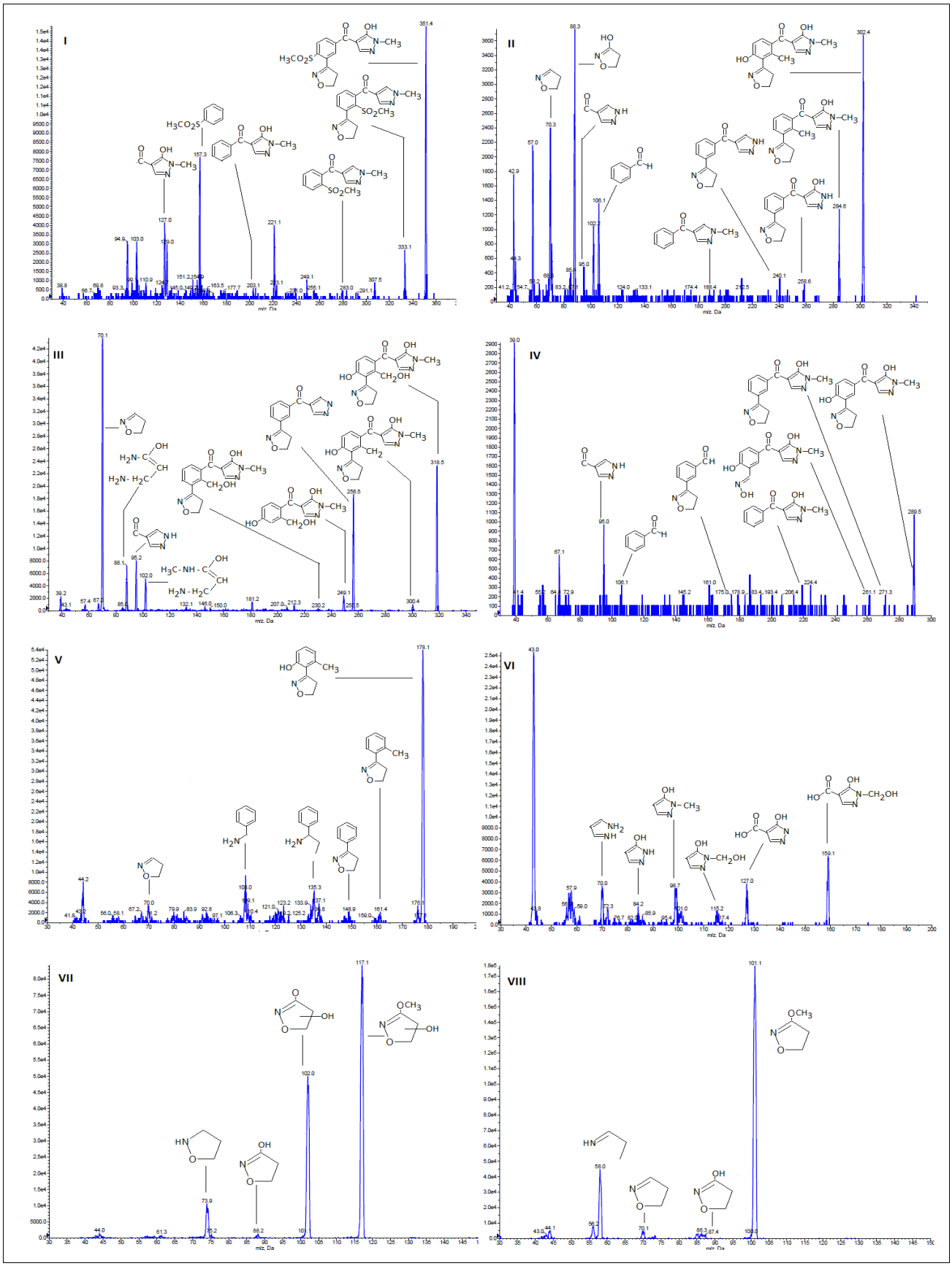

Figure 4. Proposed structures of degradation products and their fragmentation patterns on mass spectra recorded in (+) ESI mode

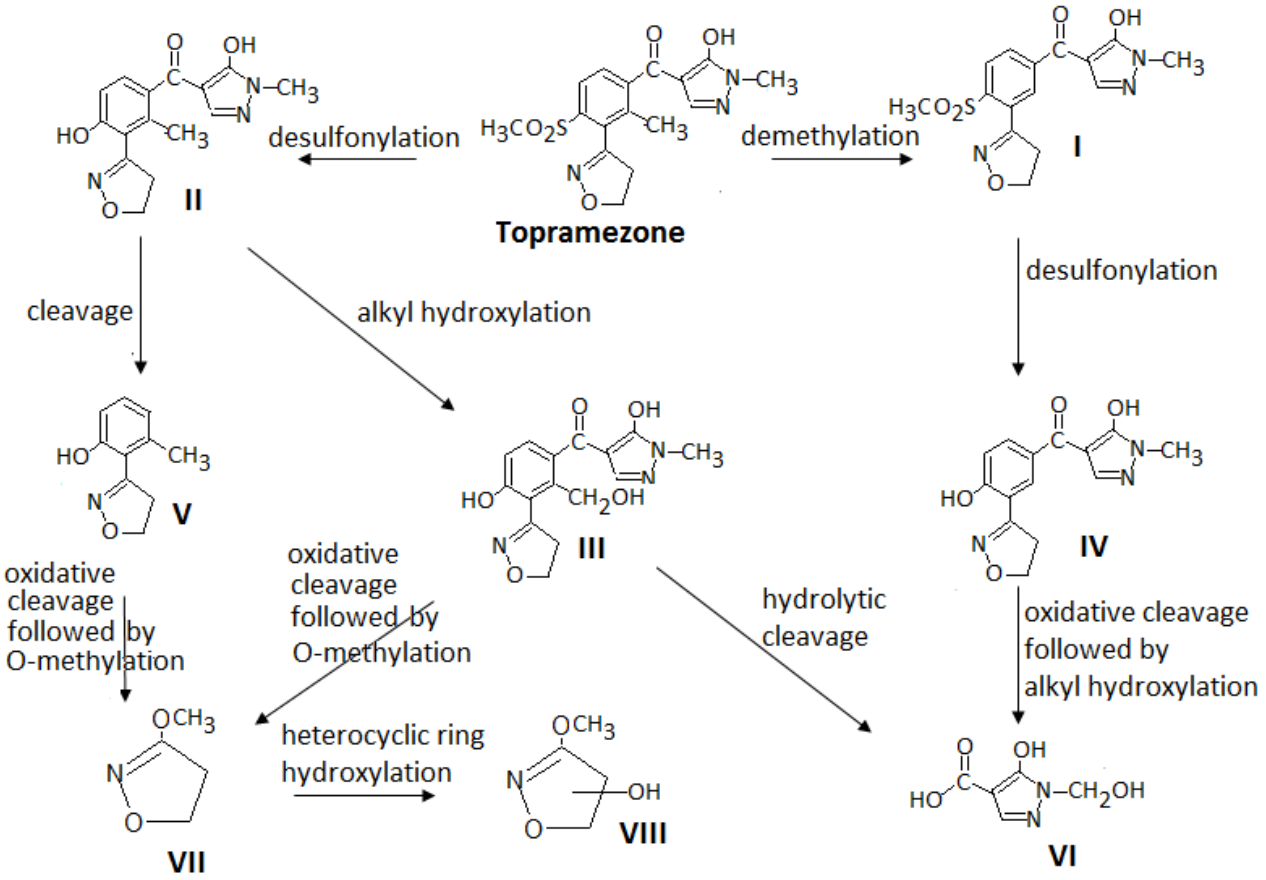

Figure 5. Proposed pathways for the degradation of topramezone by the *Trichoderma* isolate

Note: [I: [3-(4,5-dihydro-1,2-oxazol-3-yl)-4-mesylphenyl](5-hydroxy-1-methyl-pyrazol-4-yl)methanone, II: [3-(4,5-dihydro-1,2-oxazol-3-yl)-4-hydroxy-*o*-tolyl](5-hydroxy-1-methyl-pyrazol-4-yl)methanone, III: [3-(4,5-dihydro-1,2-oxazol-3-yl)-2-hydroxymethyl-4-hydroxyphenyl](5-hydroxy-1-methyl-pyrazol-4-yl)methanone, IV: [3-(4,5-dihydro-1,2-oxazol-3-yl)-4-hydroxyphenyl](5-hydroxy-1-methyl-pyrazol-4-yl)methanone, V: 2-(4,5-dihydro-1,2-isoxazol-3-yl)-4-hydroxytoluene, VI: 1-*hydroxymethyl*-5-hydroxy-4-pyrazolecarboxylic acid, VII: 1-methoxy-4-hydroxy-4,5-dihydroisoxazole, VIII: 1-methoxy-4,5-dihydro-isoxazole]

DISCUSSION

Our studies showed that incubation of the media containing topramezone, but without the fungal isolate, did not produce any significant amount of degradation of the herbicide. However, when incubated with the *Trichoderma* isolate both in the media and in soil, only about 15% of the applied topramezone persisted after 30 days of incubation.

A similar efficiency in degrading other pesticides by different strains of *Trichoderma* has also been reported. For example, *T. harzianum* 2023 rapidly degraded several organochlorine pesticides in culture media (Katayama and Matsumara, 1992). After 13 days of incubation, the amount of pesticides degraded were: DDT 20%, dieldrin 25%, endosulfan 40%, PCNB 50%, and PCP 88%. In another study, gamma ray irradiated *T. viride* and *T. harzianum* could also degraded 90% of applied Vydate®, a carbamate insecticide, in soil within 14 days of incubation (Helal and Abo-El-Seoud, 2014).

Based on our studies, the major routes of degradation of topramezone by the *Trichoderma* isolate appear to be demethylation; desulfonylation, followed by hydroxylation of the herbicides; alkyl

hydroxylation; hydrolysis of the carbonyl group of ketone; methoxylation; and hetero ring hydroxylation. These are schematically presented in Figure 5.

A demethylation process converts the herbicide into the product I through several reaction steps, the first step of which is the hydroxylation of the methyl group attached to the aromatic ring. A similar reaction was observed during the degradation of toluene by *Cunninghamella elegans* Lendner (1907), a soil-borne fungus (Prenafeta-Boldú et al., 2001). The presence of the metabolite III containing the hydroxylated methyl group is strong evidence for this transformation of topramezone. The final step for this transformation involved a decarboxylation reaction, which had not been previously for *Trichoderma* species. However, a similar decarboxylation reaction of pyrrole-2-carboxylate, catalyzed by the pyrrole-2-carboxylate decarboxylase enzyme has been previously found in two bacteria, *Bacillus megaterium* PYR2910 and *Serratia* strains (Omura et al., 1998).

A desulfonylation process of sulfonyl derivative, which involves a multi-step oxidation reaction, catalyzed by oxygenase enzymes has not been much investigated during the fungal metabolism of such compounds (Linder, 2018). However, genetic and biochemical studies in the yeast *Saccharomyces cerevisiae* suggested that the metabolism of sulfonates proceeds through a sulfite intermediate, which is akin to the metabolism of sulfonates in bacteria (Uria-Nickelsen et al., 1993; Hogan et al., 1999). An enzymatic demethylation of sulfonylmethyl by a bacterium *Pseudomonas ananatis* (AF-264684) converted mesotrione to its demethylated metabolite, a sulfinic acid derivative (Pileggi et al., 2012). It has been reported that sulfinic acid gets readily oxidized by peroxidase to its corresponding sulfonic acid (Milev et al., 2015). The sulfonic acid moiety, attached to any aromatic group, is further oxidized by oxygenase forming a hydroxylated compound (Kalme et al., 2007). In the present study, topramezone underwent a similar desulfonylation process by the *Trichoderma* isolate, forming a hydroxylated product, the metabolite II. The product IV was also most likely formed from I, through a similar process.

Another major route of topramezone breakdown was the cleavage or hydrolysis of carbonyl group bearing two ring structures, a substituted phenyl and a substituted pyrazolyl group. The carbonyl group of topramezone was cleaved or hydrolyzed generating one carboxylic group attached to the pyrazolyl ring with the formation of a substituted pyrazolecarboxylic acid (metabolite VI), and the counter part of this hydrolysis, a substituted toluene (metabolite V). Similar hydrolysis of ketonic carbonyl group has been observed in the degradation of sulcotrione and mesotrione, two other triketonic herbicides, in soil and sediment (Durand et al., 2006; Barchanska et al., 2016).

The dissociation of dihydroisoxazole moiety from topramezone, or its immediate degradation products, metabolites I, II, III, IV and V, most likely occurred through the oxidative hydroxylation on the benzene-isoxazole linkage, followed by a methylation generating metabolite VII. The latter, on ring hydroxylation, produced the compound VIII. A similar, hydrogenase enzyme-based, ring hydroxylation has been reported as a key microbial process to open a heterocyclic ring for mineralization of an aromatic N-heterocyclic compound (Yoshida and Nagasowa, 2000). Although no terminal metabolite was isolated from the isoxazole, after the ring opening, the ring hydroxylation of isoxazole of the topramezone metabolite may have provided the foundation towards the mineralization of the herbicide.

Topramezone, being a highly persistent herbicide in soil, has the potential to remain in soil for long periods and contaminate both surface and ground water resources, through leaching. However, the application of *Trichoderma* sp. to fields that have received topramezone treatments, appears to have the capacity to expedite the degradation of the herbicide and thereby, minimize the risks of topramezone residues building up in soil and leaching out to contaminate water resources.

The application of *Trichoderma* formulations, as a bio-control agent, has already been standardized in managing plant diseases, such as downy mildew in grapevine cultivars (Banani et al., 2013); damping off in tomato (Montealegre et al., 2010); dry root rot in mung bean (Dubey et al., 2009); chickpea wilt in chickpea (Dubey et al., 2007); damping off in cucumber (Roberts et al., 2005); and wilt in pigeon pea (Prasad et al., 2002). Despite such applications in the existing literature, which we reviewed, there are not many reports available on the application of *Trichoderma* strains for the purpose of bioremediation of herbicides or other pesticides in contaminated soil.

In one recent study, conducted in Indonesia, Arfarita et al. (2016) reported a promising outcome, briefly reviewed below. Their studies demonstrated a high survival of a *Trichoderma viride* strain FRP3 in

the fields, which had a history of more than 10-years of glyphosate application. In this study, the researchers used either a single or two applications of a conidial suspension (26 x 10^9 conidia g^{-1} media), in a volume of 5 L water, applied to each plot by a drip method, during a dry season (May-June 2013). While the control plots which received no additional conidial suspensions, had an indigenous microbial population of colony-forming-units CFU 0.66 x 10^6 g^{-1} soil, plots that received a single application of a conidial suspension had an increased population of microbes (CFU 8.83 x 10^6 g^{-1} soil). Plots that received two applications of the conidial suspension showed a correspondingly high microbial population with CFU of 15.97 x 10^6 g^{-1} soil. The number of colonies present in the experimental plots increased with time and correlated well with the amount of the conidia suspension of *Trichoderma viride* strain FRP3 that was introduced during the experimental period. The authors confirmed fast degradation of glyphosate using GC analysis of extracted soil samples. Within seven days after the *Trichoderma viride* strain FRP3 was applied to plots, the glyphosate content of the treated soil decreased significantly. With the single application of the conidial suspension, by the end of the experimental period of 28 days, glyphosate concentration decreased by 16 mg kg^{-1} (23.4%). In the plots that received two applications of the conidial suspension, glyphosate residues in soil decreased by 27.7 mg kg^{-1} (42.6%). When compared with the control plots, these results translated to 48% and 70% higher glyphosate degradation with a single or two applications, respectively, by the end of 28 days. The authors suggested that the fungal strain-*Trichoderma viride* strain FRP3, which had been widely used as a biological control agent in agriculture, could be used to degrade glyphosate quickly in soil contaminated with the herbicide.

The biodegradation ability of any *Trichoderma* strain would depend much on the quality of the formulation to be applied in a cropping field. Although *Trichoderma* mycelia and chlamydospores are known to have excellent biological activity, when applied to soil as suspensions, they do not survive well. Nor do they survive well during the formulation processing steps, such as drying. In contrast, the conidial suspensions are less susceptible to environmental conditions and are easily formulated (Amsellem et al., 1999; Whipps and Lumsden, 2001; Verma et al., 2005). Different, dry-flowable conidial formulations have been developed, which are quite effective in augmenting the concentrations of *Trichoderma* strains in the soil (Sriram et al., 2011; Muñoz-Celaya et al., 2012; Oancea et al., 2016; Locatellia et al., 2018).

Our studies indicate that further work is necessary to develop formulations of the *Trichoderma* strain that we isolated for field applications. Future research should focus on correctly identifying the *Trichoderma* isolate that we reported on, which can degrade topramezone fast. Following identification, research should focus on its mass culturing, to develop conidial suspensions that can be practically used in field situations.

Given that the half-life of topramezone in agricultural soil is long (about 120 days), the development and application of a beneficial fungus, such as the one we isolated, is suggested as promising to degrade topramezone from fields where the herbicide residues may have built up. Our results are suggestive of a degradation to about 50% of topramezone concentrations in soil by the *Trichoderma* isolate achievable in about 10 days. Such a development could assist in reducing the risks of topramezone residues building up in treated fields and mitigate the risks of the herbicide leaching into surface or groundwater resources.

Acknowledgements

The authors are thankful to the Director, ICAR-Directorate of Weed Research (DWR), Jabalpur, Madhya Pradesh (MP), India for granting the research facilities to perform this work. The technical advice and editorial scrutiny provided by Dr. Nimal Chandrasena and two other anonymous reviewers, which improved the MS, are greatly appreciated.

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