Indian Journal of Weed Science **51**(4): 402–406, 2019

Print ISSN 0253-8040 Online ISSN 0974-8164

Degradation of pyrazosulfuron-ethyl in the agricultural soil by *Alternaria alternata*

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Article information

DOI: 10.5958/0974-8164.2019.00084.4

Type of article: Research note

Received : 1 October 2019
Revised : 17 December 2019
Accepted : 19 December 2019

Key words
Alternaria alternata
Degradation
Herbicide
Pyrazosulfuron-ethyl

ABSTRACT

Herbicides are now used throughout the globe and a majority of applied chemicals enter into the soil to form short or long-term residues, resulting in toxicity to sensitive crops and adverse effects on human and other life forms. Therefore, present work was undertaken to isolate and identify pyrazosulfuron-ethyl degrading fungi from soil of rice field. *Alternaria alternata* was isolated and identified from rhizosphere soil of rice field, as a potent pyrazosulfuron-ethyl degrading fungus and its degradation potential was evaluated under controlled conditions. In the soil pyrazosulfuron-ethyl was efficiently degraded by *A. alternata*. Degradation of pyrazosulfuron-ethyl by *A. alternata* was achieved by the cleavage of sulfonylurea bridge and hydrolysis. *A. alternata* used in the study can effectively be used for the enhanced degradation of pyrazosulfuron-ethyl in agricultural soil or mixed with other microbial consortia for rapid degradation with half life of 7.9 days.

According to FAO (2016) estimate, an amount of 3.3 x 10^6 tones of active ingredient of pesticides is being used annually throughout the globe for management of various pests in agricultural and crop production. Use of sulfonylurea group of herbicides has been tremendously increased on a wide range of crops due to their very high herbicidal activity on broadleaf weeds and sedges in rice. This class of herbicides has low mammalian toxicities and categorized to toxicity category V (Zhu *et al.* 2002, Sondhia *et al.* 2013). Pyrazosulfuron-ethyl is frequently used in rice as pre-emergence and early post-emergence. It hampers weed growth through inhabitation of acetolactate synthase (ALS), the first enzyme that catalyzes the biosynthesis of branched amino acids valine, leucine, and isoleucine in the weeds. However, in rice, crop selectivity derives from rapid metabolism through the demethylation of the methoxy group in green plants. Some crops such as legumes and pastures are found to be very sensitive towards trace-level residues of sulfonylurea herbicides in different soils (Zhu *et al.* 2002, Sondhia 2008, Wang *et al.* 2012).

Due to large-scale commercial use of herbicides, a majority of these chemicals entered into the soil to form short or long-term residues and resulted in phytotoxicity to sensitive crops. In some studies, adverse effect of sulfonylureas to soil microbial population has also been reported (Xu *et al.* 2009). Besides this, application of herbicides including sulfonylureas in the agricultural fields also resulted in residues in surface and groundwater through runoff and leaching (Sondhia 2008, Xu *et al.* 2009).Alike other class of herbicides, sulfonylurea also detoxified in the agricultural soils mainly through microbial degradation (Huang *et al.* 2007, Sondhia *et al.* 2013, 2016).

Even though pyrazosulfuron would appear to be degraded fast in the soil, its widespread and repeated use showed adverse effects in direct-seeded and transplanted rice fields (Xu *et al.* 2009) and enhanced risk to environment and human health (Ding *et al.* 2010). In literature, degradation of pyrazosulfuron-ethyl by few bacteria such as *Pseudomonas* sp., *Pseudomonas putida*, *Acinetobacter* sp. and *Rhodopseudomonas* sp. S9-1 by Xu *et al.* (2009) and Yin *et al.* (2011) have been reported. Several fungi are known to produce enzymes that are able to degrade aromatic herbicides and have therefore been suggested as potential candidates for bioremediation (Huang *et al.* 2007, Sondhia *et al.* 2013). A large number of fungal species were found to be efficient to degrade phenylurea herbicides such as *Rhizoctonia solani*, *Bjerkandera adusta* and *Aspergillus* species

(Sondhia *et al.* 2013). Rønhede *et al.* (2005) demonstrated effective degradation of isoproturon by *Alternaria* species. But no information exists on the biodegradation of pyrazosulfuron-ethyl in the soil by *Alternaria alternata*. Therefore, in this study, degradation ability of pyrazosulfuron-ethyl in the soil by *Alternaria alternata* is reported.

Isolation of *Alternaria alternata* from rice field

Degradation experiments were done in sterilized soil under laboratory conditions. Sandy clay loam (67.32% sand, 10.00% silt, and clay 22.68%) soil with pH 7.4 and organic carbon 0.85% was collected in sterilizable polythene bags where pyrazosulfuron-ethyl was applied at a field dose of 25 g/ha continuously for three years. Samples were brought to the laboratory to isolate and identify pyrazosulfuron-ethyl degrading fungi. A commercial available formulation of pyrazosulfuron-ethyl 10% WP and analytical pyrazosulfuron-ethyl (99.5%) was purchased from United Phosphorus Limited Company, Gujarat. Analytical grade chemicals and solvents were obtained from (E Merck, Germany).

Alternaria alternata was identified in the Plant Pathology Department, JNKVV, Jabalpur. Isolation and enumeration of the fungi were done by a standard serial dilution and pour plate method. A dilution of 10^{-5} was used for inoculation of fungal media. Fungal isolates were identified and characterized on the basis of colony morphology and spore structures microscopy (Barnett and Hunter 1972). Seven days old fungal culture was maintained on sterilized potato dextrose agar (PDA) medium at 7±1°C to serve as a source of inoculums.

Degradation study of pyrazosulfuron-ethyl in the soil

The 3 mm sieved control soil (herbicide free) was air-dried and sterilized by autoclaving at 121°C for 25 minutes. For each treatment 1.0 kg of sterile soil was packed separately in sterilized polythene bags and moistened with sterile distilled water to 20% to permit good aeration. One kg of sterilized soil was inoculated with *Alternaria alternata* and incubated at 28°C for 3-days in the dark. After inoculation with fungus, herbicide was applied to the soil surface according to the treatments; soil + *Alternaria alternata* + pyrazosulfuron-ethyl at 4 mg/kg; soil + *Alternaria alternata* + pyrazosulfuron-ethyl at 8 mg/ kg; and soil + pyrazosulfuron-ethyl without *Alternaria alternata* at 4 and 8 mg/kg .

Each treatment consisted of three replications. The soil sample from each set of experiments was drawn at 0, 5, 10, 20, 30 and 60 days to determine degradation of pyrazosulfuron-ethyl in the soil.

Determination of pyrazosulfuron-ethyl in soil

For extraction and analysis of the pyrazosulfuron-ethyl, a method of Sondhia *et al.* (2013) was followed. Recovery of pyrazosulfuron from soil at 0.01, 0.5 and 1.0 µg/g levels following this method was found to be 86-92% (**Table 1**). The limit of detection (LOD) of pyrazosulfuron-ethyl was determined as the minimum concentration of pyrazosulfuron-ethyl that was detected with acceptable certainty as described by Sondhia (2008). The LOD and the Limit of Quantification (LOQ) were found to be 0.001 and 0.01 µg/mL, respectively.

Table 1. Recovery of the pyrazosulfuron-ethyl from soil at 0.5 to 1.00 µg/g fortification level with a recovery of 86 to 92% in the soil

Matrix	Fortification (µg/g)	Amount recovered (µg/g)	Recovery (%)
Soil	0.01	0.009±0.002*	90
	0.50	0.46±0.013*	92
	1.00	0.86±0.015	86

*Standard deviation

Degradation products were identified as described by Sondhia *et al.* (2013) using TLC and LC/MS-MS. Pyrazosulfuron residues in the soil were determined by UFLC with Photo Diode Array Detector. A C-18 column (ODS) of 25 mm length and 3.6 mm i.d. was used. The mobile phase acetonitrile: water (70:30) was kept at a flow rate of 0.45 ml/min. The UFLC system was standardized by injecting 20µL of standard solutions of freshly prepared pyrazosulfuron-ethyl in ACN with varying concentrations (0.001 to 10 µg/mL from a stock solution of 1000 ppm) and the detector response was measured in terms of peak areas. Areas under the peak (µV/sec) versus concentrations (µg/mL) were plotted and fit by simple linear regression to obtain an equation for the standard curve. The amount of pyrazosulfuron-ethyl in each sample was thus calculated based on the slope of the standard.

The data were calculated as mean ± S.D. and analyzed using analysis of variance technique (ANOVA). All statistical analyses were done with Excel 2003. Differences are considered statistically significant when p d" 0.05. Rates of degradation of pyrazosulfuron-ethyl in the soil was calculated by linear regression from the transformed first order rate

equation. The time of dissipation of 50 and 90 % (DT_{50}) was calculated from the equation DT_{50}= ln2/K, where K degradation rate constant.

Microbial degradation of pyrazosulfuron-ethyl in soil

After two hours (0-day) of treatment, pyrazosulfuron-ethyl residues were found to be 0.284 and 0.368 µg/g in the soil inoculated with *A. alternata* at 4 and 8 mg/kg doses, respectively. Pyrazosulfuron-ethyl residues degraded successively by the *A. alternata* and concentration decreased to 0.062 and 0.145 ìg/g after 30 days in the soil treated with 4 and 8mg/kg doses, respectively. The difference was found significant at all days and two set of treatments (p=0.05) Dissipation of pyrazosulfuron-ethyl residues by *A. alternata* at various days at different time interval is presented in **Figure 1** and **Table 2**.

Degradation parameters for pyrazosulfuron-ethyl residues were estimated in the soil inoculated with *Alternaria alternata* on the basis of first-order rate kinetics and regression equations. The degradation trends of pyrazosulfuron-ethyl residues on soil surfaces, determination coefficients, regression statistic and half-life times are shown in **Table 2** and **3**. Residues of pyrazosulfuron-ethyl in 4 and 8 mg/kg treatments, respectively degraded in the soil according to equations: y = -0.019x + 1.305 and y = -0.038x + 1.445 (linear) inoculated with *Alternaria alternata*, (**Figure 1**).

Four transformation products with [M+H]+ ions at m/z 234 (I), 198(II), 191(III) and 156 (V) were detected as a result of degradation of pyrazosulfuron-ethyl by *A. alternata* in soil and identified as ethyl 1-methyl-5-sulfamyl-1H-pyazole-4-carboxylate (I); dimethoxypyrimidin-2-ylcarbamoyl amine (II); amino sulfomyl,1-H pyrazole carboxylic acid (III) and 4,6-dimethoxypyrimidin-2-amine (IV). These were identified as based on mass spectrum data and fragmentation pattern in positive mode. Pyrazosulfuron-ethyl products at m/z 205(III) and 277(IV) were also reported from soil of rice field where pyrazosulfuron-ethyl was applied to control annual weeds (Sondhia et al. 2013). Formation of degradation products II and IV was also demonstrated by Zheng *et al.* (2008), and Wang *et al.* (2012).

Table 2. Degradation of pyrazosulfuron-ethyl in the soil by *Alternaria alternata* under laboratory conditions at two doses

Days after application	Residues (µg/g)			
	Microbial control		Chemical control	
	4 mg/kg	8 mg/kg	4 mg/kg	8 mg/kg
0	0.284±0.012(0.0)	0.368±0.073(0.0)	1.823±0.064(0.0)	2.944±0.071(0.0)
5	0.165±0.011(41.91)	0.237±0.012(35.6)	1.711±0.060 (6.14)	2.762±0.054 (6.16)
10	0.153±0.010(46.13)	0.205± 0.018(44.3)	1.349 ±0.032 (25.87)	2.1987±0.075 (25.33)
20	0.148±0.010(56.36)	0.192 ±0.096(47.83)	0.998±0.027 (45.25)	1.754 ±0.050 (40.42)
30	0.062±0.006(78.17)	0.145±0.008(60.6)	0.796±0.011 (56.33)	1.598±0.063 (45.72)
60	0.037± 0.005(86.97)	0.096±0.015(73.91)	0.679±0.011 (62.79)	1.198±0.063 (59.30)

[a]Mean of three replicates; SD: standard deviation; figures in parentheses indicate % dissipation.

Table 3. First order dissipation equation, R^2, rate constant, DT_{50} and DT_{90} values and regression statistics of pyrazosulfuron-ethyl in the soil under laboratory conditions

Parameter	Treatment			
	4mg/kg*	8mg/kg*	4mg/kg**	8mg/kg**
Rate constant (day^{-1})	3.8×10^{-2}	1.9×10^{-2}	1.7×10^{-2}	1.5×10^{-2}
R^2	0.943	0.917	0.859	0.914
DT_{50}	7.92	15.84	17.70	20.06
DT_{90}	60.57	121.15	135.41	153.46
F	28.792	184.20	14.20	27.74
Standard Error	0.006	0.001	0.004	0.003
t Stat	-5.365	-13.57	-3.76	-5.27
P-value	0.013	0.0008	0.032	0.013
K	0.038	0.019	0.017	0.015

*With fungi;**: without fungi

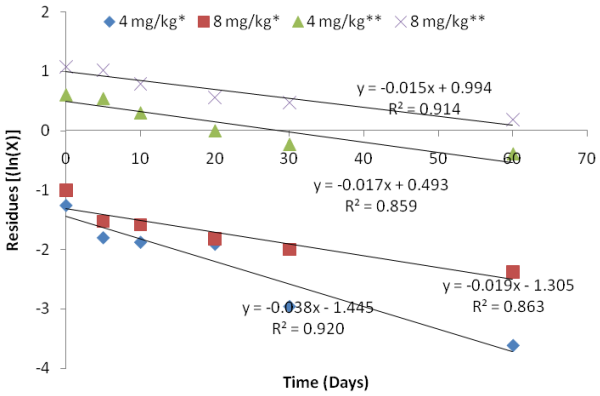

Figure 1. Dissipation kinetics of pyrazosulfuron-ethyl by *Alternaria alternata* under laboratory conditions at two doses in fungal assited tretatments, 4 mg/kg* (K, -0.038, P=0.013) and 8 mg/kg* (k, 0.019, P=0.0008) than in without fungal assited tretatments 4 mg/kg (k, 0.017, P=0.032) and 8 mg/kg** (k, 0.015, P=0.013)**

Chemical degradation of pyrazosulfuron-ethyl in the soil

In chemical degradation experiment, pyrazosulfuron-ethyl residues ranged from 1.823 to 2.944 µg/g in the soil at 4 and 8 mg/kg treatments, respectively after two hours (0-days) of treatment. Residues degraded slowly in the chemical control soil in comparison to the fungi assisted degradation experiment and reached to a concentration of 0.679 and 1.198 µg/g after 60 days in the soil treated with 4 and 8 mg/kg of pyrazosulfuron-ethyl, respectively. The difference was found significant at all days and two set of treatments (p=<0.05) Dissipation of pyrazosulfuron-ethyl residues without *A. alternata* at various days at different time interval is presented in **Table 2**.

Degradation of pyrazosulfuron-ethyl residues on soil surfaces, determination coefficients, and half-life times are shown in **Table 2**. Pyrazosulfuron-ethyl residues dissipated according to first order kinetics [(y = -0.017x + 0.493 and y = -0.015x + 0.994 (linear)] in the soil treated with 4 and 8 mg/kg of pyrazosulfuron-ethyl, respectively (**Figure 1**). The half-life of pyrazosulfuron-ethyl at 4 and 8 mg/kg of doses without fungal inoculation was found to be 17.7 and 20.06 days, however it was 7.92 and 15.84 days, respectively in the soil inoculated with *Alternaria alternata* in these treatment, which was lower in comparison to chemical assisted degradation.

Chu *et al*. (2002) reported the movement of pyrazosulfuron-ethyl in the bottom layer of soil. In addition to this, herbicide can also be lost through the process of photolysis and uptake by the plants. Under laboratory conditions, some factors such as leaching to the bottom layer, photodegradation, plant uptake *etc*, did not play any role in pyrazosulfuron-ethyl degradation and this may results in a higher half-life in the soil than the field soils.

Degradation of pyrazosulfuron-ethyl was slow in the chemical control treatments in comparison to the soil inoculated with *A. alternata* and only 62.7 and 59.3% dissipation was achieved by 60 days in both the doses. However, rapid degradation of pyrazosulfuron-ethyl (86.9 to 73.6%) was found in the treatment having *A. alternata* along with this herbicide at two doses (**Table 2**). A two-tailed test ($\alpha 2$=5%) was analyzed to the data using the T-Test for measuring differences in degradation of pyrazosulfuron-ethyl in soil with *A. alternata* and without fungi (**Table 3**). A significant difference (T-statistic -5.36, p=<0.05 and 0.05%).) was found between two sets of degradation of pyrazosulfuron-ethyl experiments in the soil. This study demonstrated potential of *Alternaria alternata* to degrade higher concentration of pyrazosulfuron-ethyl in the treated soil. Degradation products were formed as a result of cleavage and hydrolysis of the sulfonylurea bridge. It has been well demonstrated that low pH values accelerate the hydrolysis of the sulfonylurea bridge, but at pH 10, contraction of the sulfonylurea bridge may be the concise pathway. Similar findings have been demonstrated by Xu *et al.* (2009).

Microbial degradation is found to be an important mechanism of pyrazosulfuron-ethyl degradation in the soil. Soil fungi, *Alternaria alternata* was found effective to degrade pyrazosulfuron-ethyl up to 87% in the soil at tested dose under laboratory conditions. Degradation of pyrazosulfuron-ethyl was found rapid in the soil inoculated with *A. alternata* than chemical degradation. The main degradation pathway was the sulfonylurea bridge cleavage and hydrolysis. The results demonstrated that *A. alternata* used in the study can be effectively used for the enhanced degradation of pyrazosulfuron-ethyl in agricultural soil or may be mixed with other microbial consortia for rapid degradation. The feasibility of using *A. alternata* alone or in combination with other microbial consortia to detoxify pyrazosulfuron-ethyl contamination in practice needs to be investigated in detail in the future.

REFERENCES

Barnett HL and Hunter BB. 1972. *Illustrated Genera of Imperfect Fungi*, 3rd edition, Burgess Publishing Co., 273 p.

Chu C, Lin HT, Wong SS and Li GC. 2002. Distribution and degradation of pyrazosulfuron-ethyl in paddy field. *Plant Protection Bulletin* **44**: 147–156.

Ding F, Liu W, Zhang X, Wu LJ, Zhang L and Sun Y. 2010. Identification of pyrazosulfuron-ethyl binding affinity and binding site subdomain IIA in human serum albumin by spectroscopic methods. *Spectrochim Acta A* **75**: 1088–1094.

FAO (2016) FAOSTAT.inputs/pesticide use, http:// www.fao.org/faostat/en/#dataRP.

Huang X, He J, Sun JQ, Pan JJ, Sun XF and Li SP. 2007. Isolation and characterization of a metsulfuron-methyl degrading bacterium *Methylopila* sp. S113. *International Journal of Biodegradation* **60***:* 152–158.

Rønhede S , Jensen B, Aamand SRJ, Juhler RK and Aamand J. 2006 Hydroxylation of the herbicide isoproturon by fungi isolated from agricultural soil. *Applied and Environmental Microbiology* **71**(12): 7927–7932

Sondhia S. 2008. Determination of imazosulfuron persistence in rice crop and soil. *Environmental Monitoring and Assessment* **137**: 205–211.

Sondhia S.2009. Persistence of oxyfluorfen in soil and detection of its residues in rice crop. *Toxicology and Environmental Chemistry* **91**(3): 425–433.

Sondhia S, Waseem U and Verma RK. 2013. Fungal degradation of an acetolactate synthase (ALS) inhibitor pyrazosulfuron-ethyl in soil. *Chemosphere* **93**(9): 2140–2147.

Sondhia S, Rajput U, Verma RK and Kumar A. 2016. Biodegradation of the herbicide penoxsulam (triazolopyrimidine sulphonamide) by fungal strains of *Aspergillus* in soil. *Applied Soil Ecology* **105**: 196–206.

Wang Y, Dum L, Chenm Y, Lium X, Zhoum X, Tan H, Bai L and Zeng D.2012. Biodegradation of pyrazosulfuron-ethyl by *Acinetobacter* sp. CW17. *Folia Microbiologia* **57**: 139–147.

Xu J, Li XS, Xu YJ, Qiu LH, Pan CP. 2009. Biodegradation of pyrazosulfuron-ethyl by three strains of bacteria isolated from contaminated soils. *Chemosphere* **74***:* 682–687.

Yin LB, Liu Y, Zhang DY and Zhang SB. 2011. Isolation and characterization of *Rhodopseudomonas* sp. $S_{9\text{-}1}$ capable of degrading pyrazosulfuron-ethyl. *Journal of Advances in Material Research* **356-360**: 1152–1163.

Zheng W, Yates SR and Sharon KP. 2008. Transformation kinetics and mechanism of the sulfonylurea herbicides pyrazosulforon-ethyl and halosulfuron-methyl in aqueous solutions. *Journal of Agricultural and Food Chemistry* **56**: 7367–7372.

Zhu QZ, Degelmann P, Niessner R and Knopp D. 2002. Selective trace analysis of sulfonylurea herbicides in water and soil samples based on solid-phase extraction using a molecularly imprinted polymer. *Environmental Science and Technology* **36**: 5411–5420.