Current Journal of Applied Science and Technology

40(26): 3-10, 2021; Article no.CJAST.74038
ISSN: 2457-1024
(Past name: British Journal of Applied Science & Technology, Past ISSN: 2231-0843, NLM ID: 101664541)

Degradation of Pyrazosulfuron Ethyl in Rice Ecosystem by *Aspergillus terreus*

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Authors' contributions

This work was carried out in collaboration among all authors. Author SSM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PPC and SRM managed the analyses of the study. Author AR managed the literature searches and edited for publication. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/CJAST/2021/v40i2631519
Editor(s):
(1) Dr. Elena Lanchares Sancho, University of Zaragoza, Spain.
Reviewers:
(1) P. Janaki, Tamil Nadu Agricultural University, India.
(2) Ahmad Ibrahim, Federal University Lokoja, Nigeria.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74038

Original Research Article

Received 20 July 2021
Accepted 28 September 2021
Published 05 October 2021

ABSTRACT

Investigation on the degradation of pyrazosulfuron ethyl by *Aspergillus terreus* in the rice ecosystem was conducted at the Department of Biochemistry, UBKV, Pundibari Coochbehar, West Bengal, India. The soil was collected from the paddy field to isolate fungal inoculums. Results revealed that *Aspergillus terreus* can survive in minimal broth containing pyrazosulfuron ethyl at 1000 ppm and degrades through two major pathways, first involves the cleaves of sulfonylurea bridge resulting in the formation of two major metabolites viz., 2-amino-4, 6 –dimethoxyprimidine, 5-aminosulfonyl-1-methyl-pyrazole-4-carboxylic ethyl ester and, second was the cleavage of sulfonyl amide linkage which forms the metabolite viz; 1 methyl pyrazole-4-carboxylic acid ethyl ester,1 methyl pyrazole -4-carboxylic acid, 5-carbamoyl -1-methyl pyrazole -4-carboxylic acid ethyl-ester. The enzyme involved in these transformations can be utilized to decontaminate soil and water from Pyrazosulfuron ethyl residue. Even, the gene responsible for the production of these useful enzymes can be exploited for future research.

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Keywords: Aspergillus terreus; pyrazosulfuron ethyl; herbicide; rice ecosystem.

1. INTRODUCTION

Sulfonylureas are new class of herbicides characterized by low application rate, having high herbicidal activity, broad action spectrum, low toxic, used on wide range of crops, such as rice, wheat, barley, corn, cotton, potato, and soybean [1,2,3].The application of these herbicides kill the weeds in 3-5 week [4]. Pyrazosulfuron ethyl is a popular recent origin Sulfonylureas class of herbicides, widely used in rice for pre-and post-emergent control [5] of weeds. Due to frequent use of these herbicides, a majority of these chemicals entered in to soil to form short or long term residues and resulted in phytotoxicity to sensitive crops [6]. There are few studies which report adverse effect of sulfonylureas to soil microbial population. [7]. Like other herbicides sulfonylurea also degrades in soil through microbial degradation [8,9,10,11]. Although pyrazosulfuron are reported to be fast degraded in the soil [6] but its frequent use showed adverse effect on paddy production and enhanced risk to environment and human health [6]. There are few reports on bacterial degradation of Sulfonylureas in to soil [7,12]. Degradation of chlorsulfuron and metasulfuron methyl by *Streptomyces griceolus* bacteria [13], [14] and Trisulfuron by *S. griseolus* [15] have been well established. Like bacteria, fungal species are also found to be efficient to degrade phenylurea herbicides, but, a little study has been done on Pyrasosulfuron -ethyl degradation by fungi in the context of the ecological risk of its long term application. *Aspergillus terreus* is considered as an important pyrazosulfuron ethyl degrading fungi [16]. In this article, we described the isolation of the new Pyrazosulfuron ethyl degrading fungi, Aspergilus terreus and studied the Pyrazosulfuron ethyl degradation in contaminated soil. The metabolites formed during the biodegradation of Pyrazosulfuron ethyl were also analyzed.

2. MATERIALS AND METHODS

2.1 Chemical

A sample of technical grade Pyrazosulfuron ethyl (90.5%) and analytical Pyrazosulfuron ethyl (99.9%) were obtained from United Phosphorus Limited Company, Mumbai. Technical grade Pyrazosulfuron was further purified by recrystallisation. The purity was checked by Thin layer chromatography (TLC) (solvent system - chloroform: acetonitrile = 2: 3, v/v, R_f =0.35). It is a white amorphous solid. Laboratory grade reagents and solvents were locally procured, purified and used. All the solvents were distilled and dried before use.

2.2 Equipments

Autoclave, BOD incubator, laminar air flow, mechanical shaker, pH meter, vortex mixture, compound microscope, thin layer chromatography (TLC) applicator, Liquid chromatography with tandom mass spectrometry(LC-MS/MS), gas chromatography-mass spectrometery (GC-MS/MS) and Vacuum drier.

2.3 Preparation of Soil Suspension

A sample of 1.0 g of soil collected from rice field of research farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, North Bengal, was suspended in 100 ml distilled water in a conical flask. The flask was thoroughly shaken to make uniform soil suspension to use as inoculums for soil fungi.

2.4 Isolation of Pyrazosulfuron Degradative Microorganisms from Soil Samples

Minimal media (100 ml) of fungi was taken in 250 ml sterilized conical flask. The minimal media (a culture medium for microorganisms that contains the minimal necessities for growth) is prepared with one fifth of the strength of original fungal media. Required quantity of Pyrazosulfuron ethyl was added to minimal broth media in order to get 100 ppm, 250 ppm, 500 ppm, 1000 ppm of active ingredient. Similarly, distilled water containing mineral solution was also taken in conical flask and pyrazosulfuron ethyl was added to get final concentration of 1000 ppm. From each category, 1.0 ml of soil suspension was added to each flask as inoculums. All the flasks were incubated at room temperature for a long period (65 days) with a view to enrich the herbicide tolerant microorganisms. For isolation of Pyrazosulfuron ethyl tolerant/transforming microorganisms, enriched culture from each flask was streaked on media of fungi amended with 100 ppm pyrazosulfuron ethyl [17]. The colony grown in 1000 ppm conc. of pyrazosulfuron ethyl in water

plus minerals media was considered as resistant one for fungi. It was taken out after 65 days, purified by serial dilution and stored for taxonomic identification.

2.5 Identification of Isolated Fungus from Soil

For fungal identification used stain such as lacto phenol/cotton blue mounting procedure: A drop of lacto phenol or cotton blue was placed on a clean, dust free slide. A small tuft of the fungus preferably with spores and spore bearing structures was transferred onto the drop under aseptically environment and was gently teased by the sterile mounted needle. After proper mixing of the fungal material a cover-glass was placed over the preparation taking care to avoid trapping air bubble in the stain.

2.6 Degradation of Pyrazosulfuron ethyl by Isolated Fungus and Isolation of Metabolites in Broth Media

1ml broth media of the isolated soil fungi was transferred to 100 ppm. Pyrazosulfuron ethyl conc. in a conical flask of fungi media for incubation at room temperature.The growths of the soil fungus in the above flask were checked by drowing 1 ml from it and pour plating in petriplate. In order to asses further tolerant of the fungus to Pyrazosulfuron ethyl 1ml of broth was drown from the 1st flask (100ppm PSE conc.) and transferred to flask of 250 ppm pyrazosulfuron ethyl concentration. It gives growth visualization in 30 days. Similarly the flasks which carry 500 & 1000 ppm pyrazosulfuron ethyl were also inoculated. These flasks were incubated up to 50 days and 21 days, respectively. The growth of the fungus bacteria inoculated was periodically checked by visual observation and pour plating. A fungus grown in 1000 ppm conc. of pyrazosulfuron ethyl in water plus minerals media was considered as resistant one. It was taken out after 21 days. During these phenomena pyrazosulfuron ethyl was degraded in to several components. This component was studies by chromatographic technique.

2.7 Component Separation of Fungal Degraded

Growth minimal media of 100 ppm, 250 ppm, 500 ppm and 1000 ppm water medium in fungus of each soil samples were filtered separately. The bunch of microbe was drowning out. Balance solution was extracted first with chloroform solvent then ethyl acetate solvent by gravity separator funnel. Pyrazosulfuron ethyl component were soluble in the solvents. Similarly ethyl acetate extract was also extracted by gravity separation method to finding the degraded product of metabolites.

2.8 Extraction and Characterization of Metabolites from Broth and Soil Product

Degraded products were extracted from broth in different time interval by partitioning in chloroform. The incubated soil was also extracted in ethyl acetate by continuous shaking for 4 hours and filtered. Solvent was then evaporated under low pressure in rotary vacuum evaporator to obtain a crude mixture of products. Products were purified and characterized by the following chromatographic and spectroscopic techniques.

2.9 Chromatography

2.9.1 Preparative thin layer chromatography

Silica gel based TLC plates were prepared by spreading a slurry of silica gel G containing 10 per cent binder (gypsum) and 1g fluorescence in water on 20cm X 20cm glass plates, uniformly maintaining a thickness of 0.75mm using a Thin layer chromatography (TLC) applicator. Prepared plates were air dried first and then activated at 120^{0}c for 2 h. The sample solutions were spotted on the TLC plates using capillary tubes. Spots were visualized under UV light, marked and scrapped. The scrapped silica gel was extracted with mobile phase solution (40% acetone in Hexane).

2.9.2 Liquid chromatography mass spectroscopy

An API 3200 Qtrap mass spectrometer hyphenated to Shimadzu UFLC was used to mass characterization of degraded products. Mass spectrometric analysis was performed with electro spray ionization (ESI) in positive (5500 eV.) mode for each sample. The nebulizer gas and heater gases were adjusted at 30 psi and 55 psi, respectively. The ion source temperature was set at 500^{0}C. Each sample was injected by infusion technique at the rate of 10 μLs^{-1}.

2.9.3 Gas chromatography mass-mass spectroscopy

Varian Saturn 2200 GC/MS with the CP-3800 and 2000 Series Ion Trap MS was used for the separation and characterization of degraded products.

3. RESULTS AND DISCUSSION

3.1 Isolation and Characterization of Soil Fungus

The media incubated with Pyrazosulfuron ethyl showed a rapid growth in colonies of fungus, appeared as a brown cinnamon with various shades. Microscopic observations (X 100) showed columnar like structure having various conidiophores, with smooth and colorless hyalines. Conodia was measured 1.5-2.3 µm diameter by ocular microscope and appeared as slightly elliptical and strait. A special property was seen in this genera that globose was stitch on hyphae .Thus from above characters it was concluded that the isolates of farm soil, contained the fungus *Aspergillus terreus* [18,19].

3.2 Degradation of Pyrazosulfuron-Ethyl by *Aspergillus terreus*

Isolated fungus *Aspergillus terreus* degrades the Pyrazosulfuron ethyl in incubated media, formed five metabolites, designated as compound F_1, F_2, F_3, F_4 & ,F_5 (analyzed by GC-MS/MS and LC-MS/MS and characterized by their mass ions and fragmentation patterns).Pyrazosulfuron ethyl degrades through two major pathways, first involves the cleavage of sulfonylurea bridge resulting in the formation of two major metabolites viz; 2- amino- 4, 6– dimethoxy-primidine(F_5),5-aminosulfonyl-1-methyl-pyrazole-4-carboxylic ethyl ester(F_1) and, second was the cleavage of sulfonylamide linkage which forms the metabolite viz;1 methyl pyrazole-4-carboxylic acid ethyl ester (F_2), 1 methyl pyrazole -4-carboxylic acid (F_3) and 5-carbamoyl-1-methyl pyrazole-4-carboxylate (F_4). The metabolites formed was separated and characterized by GC-MS/MS and mass characterization was performed by LC-MS/MS through mass ions and fragmentation patterns which are given below:

***Compound F_1*:** m/Z 206 with the Compound F_2 showed a molecular ion peak (MH^+) at m/Z 234.The loss of $-C_2H_4$ radicals from the molecular ion peak gave a peak at m/Z 206. A fragmentation at m/Z 188 was generated from elimination of molecular H_2O. Hence from the mass fragmentation pattern the structure of F_1 may be elucidated as 5-aminosulfonyl-1 –methyl pyrazole-4- carboxylic acid ethyl ester This suggested that the primary step of the degradative route is the cleavage of sulfonylurea bridge (Fig. 1). According to previous reports, the cleavage of sulfonylurea bridge is the common degrading route of sulfonylurea herbicides as reported for the degradation of sulfonylureas by bacteria and fungi [20,21].

***Compound F_2*:** F_2 showed a molecular ion peak (M^+) at m/Z 154 together with diagnostic peak at m/Z 139, 125, 109 and 81 by the elimination of radicals $-CH_3$, C_2H_5, $-OC_2H_5$, and $CO_2C_2H_5$ respectively from (M^+).Hence considering the above mass fragmentation pattern compound F_2 to be assigned as 1- methyl pyrazole -4-carboxylic acid ethyl ester (Fig. 2).

***Compound F_3*:** Molecular ion peak of F_3 (M^+) at m/Z 126 and other two fragments m/Z 109 (M^+ -OH), m/Z 81(M^+ -COOH) revealed that the structure of F3 may be elucidated as 1-methyl pyrazole -4-carboxylic acid (Fig. 3).

***Compound F_4*:** This compound showed a molecular ion peak (M^+) at m/Z 197. The ionic fragment at m/Z 182 could be resulted through the loss of NH_2 radical and addition of hydrogen radical from M^+. A peak at m/Z 153 was formed by the elimination of –COH radical from m/Z 182. The peak at m/Z 81 was formed from m/Z 153 through the simultaneous elimination of $–CH_3$. (m/Z 138), CH_2 (m/Z 124), -O (m/Z 108) and –CO (m/Z 81). Finally at peak at m/Z 68 was formed by the elimination of CH_3 radicals from m/Z 81. Analysis of all the fragments the structure of F_4 was assigned as ethyl 5-carbamoyl-1-methyl-1-pyrazole-4-carboxylate (Fig. 4).

***Compound F_5*:** The molecular peak at m/Z 156 (MH^+) of F_5 may be assigned as 2-amino- 4,6-dimethoxy - pyrimidine by the analysis of mass spectral data m/Z 140 (MH^+ -NH_2),m/Z 124 (m/Z 140 –CH_4 radical) and m/Z 100 (elimination of 24 atomic mass unit), Fig. 5.

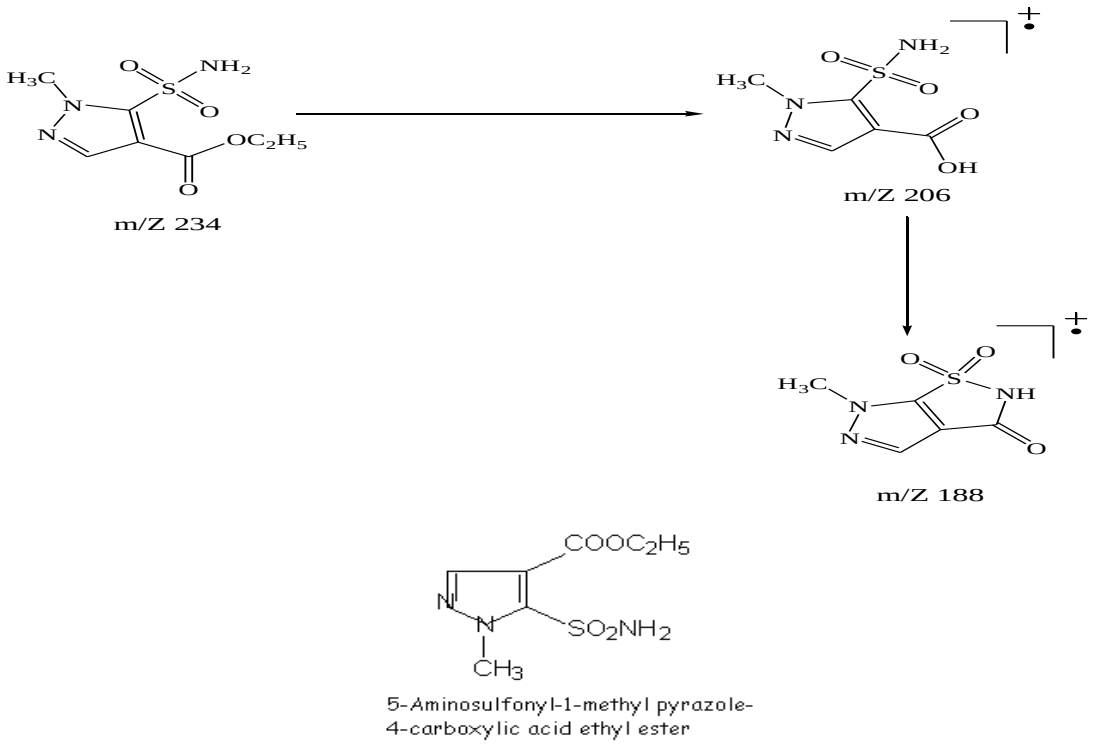

Fig. 1. Mass fragmentation pattern of compound F_1 with elucidated name

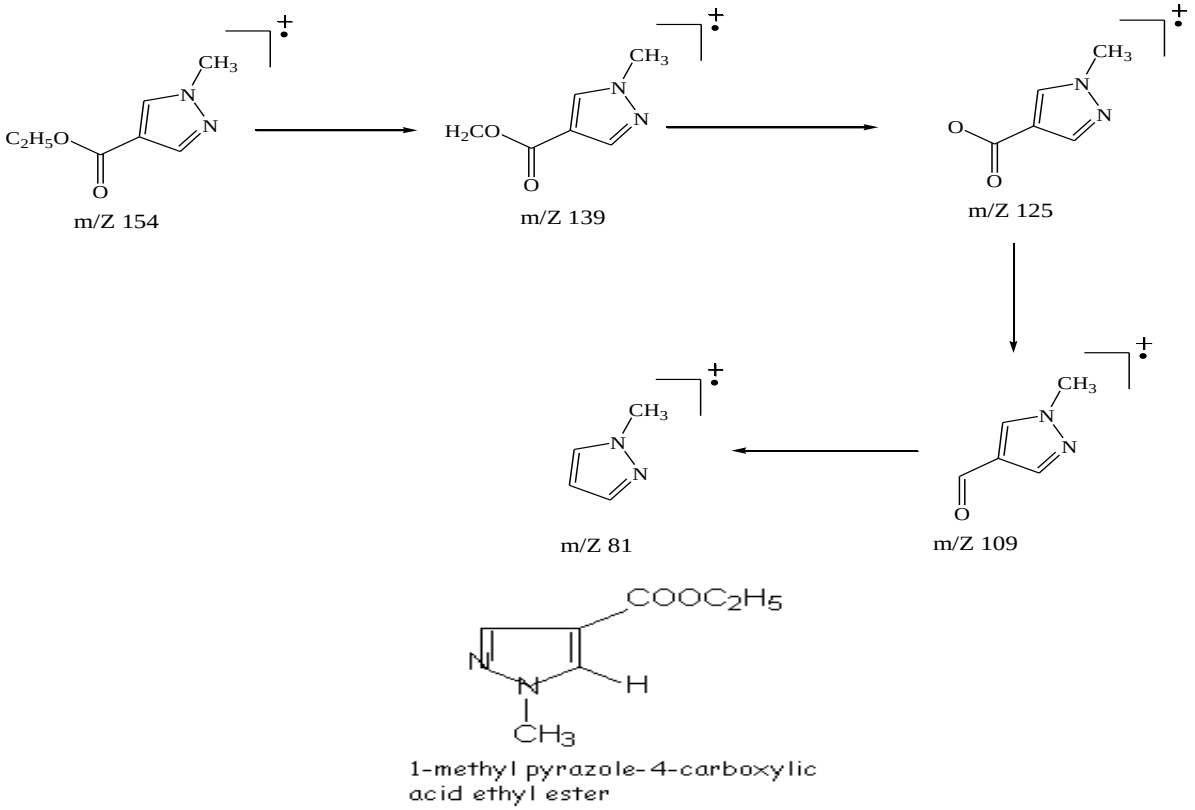

Fig. 2. Mass fragmentation pattern of compound F_2 with elucidated name

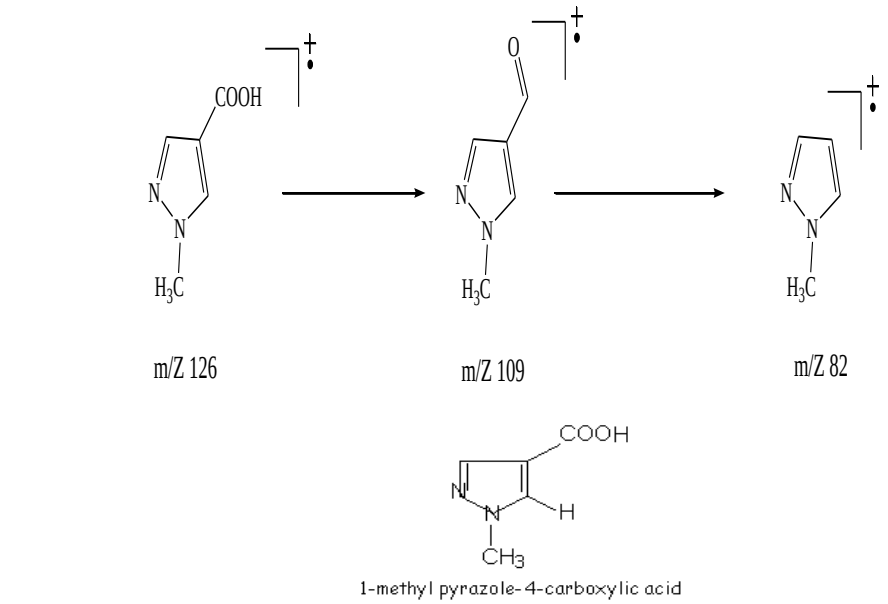

Fig. 3. Mass fragmentation pattern of compound F_3 with elucidated name

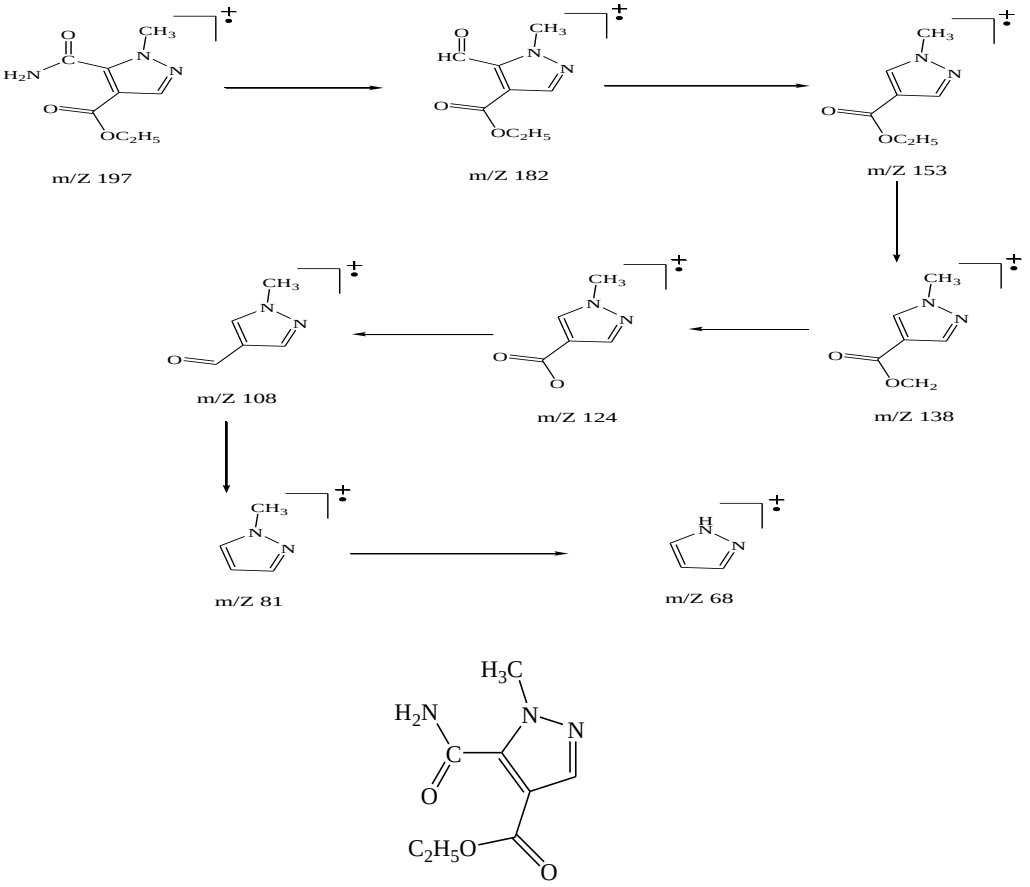

Fig. 4. Mass fragmentation pattern of compound F_4 with elucidated name

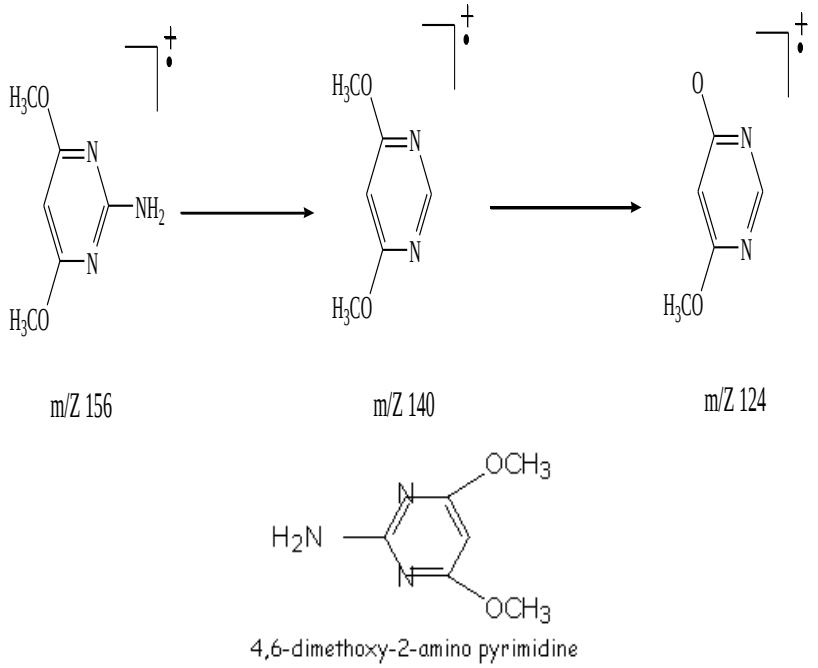

Fig. 5. Mass fragmentation pattern of compound F_5 with elucidated name

4. CONCLUSION

The isolated Pyrazosulfuron ethyl degrading fungi (*Aspergillus terreus*) can survive in minimal broath containing pyrazosulfuron ethyl at 1000 ppm and degrades the herbicide by harvesting energy through two major pathways. One path involves the cleavage of sulfonylurea bridge resulting in the formation of two major metabolites, first involves the cleavage of sulfonylurea bridge resulting in the formation of two major metabolites viz., 2- amino-4,6–dimethoxyprimidine (F_5) , 5-aminosulfonyl-1-methyl-pyrazole-4-carboxylic ethyl ester (F_1) and, second was the cleavage of sulfonyl amide linkage which forms the metabolite viz; 1 methyl pyrazole-4-carboxylic acid ethyl ester (F_2),1 methyl pyrazole -4-carboxylic acid (F_3) and 5-carbamoyl-1-methyl pyrazole-4-carboxylate (F_4). On the basis of results obtained, it can be concluded that the enzyme involved in these transformations can be utilized to decontaminate soil and water from Pyrazosulfuron ethyl contamination. Even, the gene responsible for the production of these useful enzymes can be exploited for future research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brown HM. Mode of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic Science. 1990;29:263-812.
2. Zhou QY, Liu WP, Zhang YS, Liu KK. Action mechanisms of acetolactate synthesis-inhibiting herbicides. Pestic Biochem Phys. 2008;89:89-96
3. Ma Ping Ji, Wang Zhe, Lu Peng, Wang jie Hui, Ali Wasim Shinawar, Li Peng-Shun,Huang Xing. Biodegradation of the sulfonylurea herbicide chlorimuron-ethyl by the Strain Pseudomonas sp. LW3. FEMS Microbiology Lett. 2009;296(2):203-09.
4. Zanardini E, Arnoldi A, Boschin G, Agostina A.D, Negri M, Sorlini C. Degradation pathways of chlorosulfuron and metasulfuron-methyl by a Pseudomonas fluorescence strain. Annals of Microbiology. 2002;52:25-37.
5. Yamamoto S, Nawamaki T, Wakabayashi T, Kasai BY. Development of a new rice herbicide, pyrazosulfuron ethyl. J. Pestic Science.1996;21:259-68.
6. V. Ezhilarasi, P. Janaki, P. Murali Arthanari and C. Chinnusamy. Dissipation and fate of ready mix combination of pyrazosulfuron-ethyl and pretilachlor in rice field. Indian Journal of Weed Science. 2018;50(3):250–56.

7. Xu J, Li XS, Xu YJ, Qiu LH,Pan C.P. Biodegradation of pyrazosulfuron-ethyl by three strains of bacteria isolated from contaminated soils. Chemosphere. 2009; 74:682-87.
8. Huang X, He J, Sun JQ, Pan JJ, Sun XF and Li SP. Isolation and characterization of a metasulfuron–methyl degrading bacterium Methylopila sp.S113. International Journal of Biodegradation. 2007;60:152-58.
9. Sharma Seema, Kaushik Banerjee, Partha P Choudhury. Degradation of chlorimuron-ethyl by Aspergillus niger isolated from agricultural soil. FEMS Microbiology Lett. 2012;337:18–24.
10. Brown HM, Joshi MM, Van AT, Carski T.H, Dulka JJ, Craig PM, Reisser RM, Livingston RS, Doughty J .Degradation of thifensulfuron methyl in soil: role of microbial carboxy esterase activity. J Agr food Chem. 1997;45:955-61.
11. Harvey J, Dulka JJ, Anderson JJ. Properties of Sulfometuron methyl affecting its environmental fate: aqueous hydrolysis and photolysis, mobility and adsorption on soils, and bioaccumulation potential. J Agric Food Chem. 1985;33: 590–96.
12. Yin LB, Liu Y, Zhang DY and Zhang SB. Isolation and characterization of Rhodo pseudomonas sp.S9-1 capable of degrading pyrazosulfuron-ethyl. Journal of Advances in material Research. 2011; 356-360:1152-63
13. Sabadie J. Alcoholysis and chemical hydrolysis, of chlorsulfuron. Weed Res 1990;30:309–16.
14. Sabadie J. Hydrolyze chimique acid de metasulfuron methyl. Weed Res. 1991;31: 413 -41.
15. Dietrich RF, Reiser RW, Stieglitz B. Identification of microbial and rat metabolites of triflusulfuron methyl, a new sugar beet herbicide. J Agric Food Chem.1995;43:531-36.
16. Peeples KA, Moon MP, Lichtner FT, Wittenbach VA, Carsky TH, Woodword MD, Graham K, Reinke H. DPX66037 a new low rate sulfonyl urea for post emergence weed control for sugar beet and fodder beet. Weeds. 1991;2:25 – 29.
17. Martin S. Paul, Rinaldo. Sites of the reserve phase: Pine Lawn Valley Western New Mexico. Fieldiana, Anthropology. 1950;38(3):403-05, 407-17, 19-33, 35-41, 443-577.
18. Raper BK, Fennell DI. The Genus Aspergillus. Baltimore: Williams and Wilkins Co. Edinburgh: E. & S. Livingstone. 1965;9:686
19. Balajee SA. *Aspergillus terreus* Complex, Medical Mycology. 2009;47(1): 542-46.
20. Bosehin G, Agostina AD, Arnoldi A, Marotta E, Zanardini E, Negri M ,Valle A, Sorlini C. Biodegradation of chlorosulfuron and metasulfuron-methyl by *Aspergillus niger* in laboratory conditions. Environ Sci Heal. 2003;B38:737-46.
21. Yu YL, Wang X, Luo My, Yang JF, Yu JQ, Fan DF. Fungal degradation of metasulfuron-methyl in pure cultures and soil. Chemosphere. 2005;60:460-66.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74038