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Impacts of Some Conventional Rice Herbicides on Catabolic Activity of Soil Microorganisms

[1]Reza Valiolahpor, [2]Amir Lakzian, [1]Hassan Barari, [1]Shaban Ali Mafi,
[1]Syyed Alireza Dalili and [1]Hossein Barai

[1]Agricultural and Natural Resources Research Centre of Mazandaran, Sari, Iran
[2]Ferdowsi University of Mashad, Mashad, Iran

Abstract: Conventional rice herbicides including Anilofos+ethoxysulfuron, Oxadiargyl, Thiobencarb, Cinosulfuron and Butachlor were used at a filed which had never been planted under rice, in 2006 and 2007 in Mazandaran province in north of Iran. Recommended field rates were used for all herbicides in three replications in a Randomized complete block design. Microbial biomass and microbial catabolic (richness and evenness) were determined at three intervals (15 hours, 30 and 60 days after herbicide application (DAHA). Microbial biomass was unchanged at expected field concentration, regardless of any used herbicides. Our findings suggest that using conventional herbicides at field rate have little or no effect on soil microbial communities in rice fields. In the first harvest, however, Butachlor's Shanon index (0.5± 0.8) was 30% lower than Anilofos + ethoxysulfuron, but not significant ($p \leq 0.05$). The difference between higher and lower Shanon index in the second and the third harvests were 64% and 46% lower, respectively. Though the changes of catabolic evenness were inconsistent for different herbicides, there were not any meaningful differences between treatments at any harvest.

Key words: Catabolic evenness · Shanon index · Herbicide · Microbial biomass

INTRODUCTION

Chemicals for crop protection and pest control known as pesticides are being used to ensure the production of enough supplies of food, fiber and protection of human and livestock health [1]. Herbicides are one of the major groups of pesticides, which provide a tool that man can use to promote and sustain his preferred status on earth in a number of ways. They contribute to the increased and economical production of plant and animal products, to minimize human toil in agricultural production and to the positive management of our environment for increasing benefit of mankind. Weeds have been a persistent problem in rice since the beginning of settled agriculture. For Asia as a whole, weeds cause an estimated 10-15% rice yield loss equivalent to about 50 million tons of rough rice annually [2]. Different herbicides with diverse mode of action have been used in Iranian rice field for 35 years [3]. In these days, herbicides including Butachlor, Thiobencarb, Oxadiargyl, Anilofos + ethoxysolforon, Oxadiazon and others are being used in Mazandaran provience rice filed in north of Iran, which has an area of about 230000 ha of rice field.

Unwanted side effects on non-target organisms are an environmental concern with the use of xenobiotic compounds. It is generally recognized that the microbial biomass is the eye of the needle through which all the soil microbial biomass represents only a small fraction of the total amount of soil C, N, P and S, it has a relatively rapid turnover [4]. In soil-microorganism-plant system, microbes like bacteria, fungi, algae, protozoa and some nematodes have a vital role in maintaining the soil productivity. Herbicides and pesticides affect different soil microbial processes [5-7]. So the soil microbial biomass is considered an active nutrient pool to plants. Thus anything that disrupts microbial activity in soils could be expected to affect the long-term soil productivity and would have serious consequences. Pesticides depending upon type and dose of application can alter the biomass quantitatively and qualitatively in both the short and long-term [2]. Microbial biomass

Corresponding Author: Reza Valiolahpor, Agricultural and Natural Resources Research Centre of Mazandaran, Sari, Iran, P.O. Box: 48175-556, Tel: +98(0)1243453160.

can be highly responsive to herbicide application, although, is influenced by herbicide concentration or weed levels.

At high rates of 2,4-D or glyphosate (20 and 60 g/kg, respectively) the microbial population decreased immediately after application and showed signs of brief proliferation at 5 days after application, followed by a slow increase from day 16 onward for 2,4-D and a decreasing trend for glyphosate [9]. In a review, [10] showed a negative correlation between herbicide dose and microbial biomass which dose and microbial biomass which became more marked as temperature and moisture conditions became unfavorable. Tyuryukanova *et al.* [11] reported that herbicides decreased microbial biomass in soil. Also in tilled field plots, Wardle and Parkinsone [12,13] applied two post-emergence herbicides (glyphosate and 2,4-D) at field rate and found that these chemicals affect peculiar variables associated with microbial biomass. But, glyphosate did not influence any of the microbial variable tested and addition of 2,4-D significantly influenced all microbial variables investigated, but these effect were temporary, being detectable only within the first 1-5 days of herbicide application. Growth of the microbial biomass may be enhanced by some herbicides (e.g. glyphosate), due to the herbicide acting as a nutrient source [14]. The presence of metsulfuron-methyl in the soil proved to increase the microbial biomass [9].

There is little known about the role of soil microbial diversity in the functioning of soils. Little is known on herbicide's effect on microbial community structure (the abundance and proportion of distinct phyllogenetic or functional groups) [15]. An important benefit of biological diversity to soil functioning may be to provide greater resistance to stress and disturbance [16]. Functional diversity is a component of total diversity in soil that possibly provides a more practical and ecologically relevant amount of microbial diversity [17]. This component of diversity is different from that obtained by measuring species diversity in that it concerns the range and evenness of functions expressed in situ by the microbial community, rather than the species present in soils. Microbial functional diversity includes a vast range of activities including: nutrient transformations, decomposition, plant growth promotion/suppression and modification of soil physical processes [18, 19]. A subset of this diversity can be characterized by measurement of catabolic response profiles (CRPs) [20, 21]. This approach directly assesses the catabolic diversity of microbial communities involved in decomposition activities by adding a range of simple organic substrates directly to soil and measuring the short-term catabolic responses [20, 21]. Catabolic evenness, a component of microbial functional diversity defined as the uniformity of substrate use, can be easily calculated from the CRPs.

The catabolic diversity approach provides an easily interpretable, practical indicator of the diversity component of the soil micro biota [22]. The catabolic diversity of culturable bacteria can be less in soils subjected to stress imposed by heavy metals [23-25] or disturbance associated with fumigation or heat treatments [26].

We hypothesized that application of different herbicides will have different effect on microbial processes. Our objective was to determine whether conventional rice herbicides result in short-term changes, either deleterious or beneficial, in paddy soil microbial communities.

MATERIAL AND METHODS

This experiment was carried out in 2006 and 2007 at Gharakhil agricultural station located at 56.18° longitude and 36.28° latitude. The soil was loam silt of pH 7.6. Six herbicides including, [1-Butachlor (5 l/ha), 2-Thiobencarb (Saturn) (5 l/ha), 3-Anilofos + ethoxysulfuron (Sunrice plus) (3 l/ha), 4-Oxadiargyl (3 l/ha), 5-Cinosulfuron (150 g/ha) and 6-check plot (without herbicide) consisted the treatments of the experimental design in a randomized complete block design. Three replications of each treatment were included. The plot size was 4×4 meter and was selected in field rice that no herbicide had been used previously. Nursery was prepared and manually transplanted in late June when plants were 15-18 centimeter long. For preventing combination of herbicides in neighboring plots, each plot was isolated with nylon on the ridge. Irrigation was done manually and tried to prevent spillage. Controlling of stem borer and blast was done twice and once, respectively with Rigent (trade name) granule and Hinozan (trade name). Three harvests were done on 2, 30 and 60 days after herbicide application. In each harvest 3 sub-samples were selected from each plot and then mixed well to make one sample for each plot.

Microbial biomass C was estimated using the substrate induced respiration method, as described by Sparling (1995). Briefly, 2ml of 75 mM glucose solution was added to soil equivalent to 1 g oven-dry weight in McCartney bottles, sealed with a vacutainer stopper and

incubated at 25°C for 4 h. Microbial biomass C (MBC) was calculated from the glucose-induced respiration rate (corrected for CO2 evolved in bottles with only deionised water added) as MBC (μg C g^{-1} soil) = 50.4 × respiration rate (μg CO_2 g^{-1} soil h^{-1}).

Microbial catabolic/diversity was assessed by adding a range of simple organic compounds to soil and measuring the short-term respiration responses [27, 20]. Solutions (2ml) of 16 organic substrates (as pH adjusted solutions, see below) were each added to 1 g (dry weight equivalent) of slurry soil in McCartney bottles (27.7 ml). Deionised water (2 ml) was added to additional soil samples to determine respiration in no amended soil and to confirm that the responses to substrates were greater than background respiration. All the bottles were sealed with a Vacutainer stopper and incubated at 25°C for 4 h in darkness. The bottles were shaked manually immediately after substrate addition, at 1-2 h and 10 min before measurement of respiration response [28]. At 4 h, respired CO_2, in the headspace of each bottle was determined by taking a 1 ml syringe sample and analysing $C0_2$ concentration using an infra-red gas analyzer (Model ISC 2003). The substrates consisted of one amines (L-glutamine), five amino acids (L-arginine, L-asparagine,, L-histidine, L-lysine, L-serine), two carbohydrates (D-glucose, froktose) and seven carboxylic acids (L-ascorbic acid, citric acid, glutaric acid, a-ketoglutaric acid, a-ketovaleric acid, tartaric acid and oxalic acid). The amines and amino acids were added at 10 mM, whereas the carbohydrates were added at 75 mM and the carboxylic acids were added at l00mM [28]. These rates generally gave maximum respiration responses across a range of NZ soils [28]. All solutions were adjusted to pH 5.8- 6.2 using HC1 or NaOH at the time of preparation [27] to avoid any substrate-pH effects on microbial communities.

Catabolic diversity consists of both the richness and evenness of the use of substrates [21, 17]. In this case, richness is the number of substrates metabolized by the soil microbial community, whereas evenness is the variability in substrate use [21]. Catabolic evenness (E) was calculated from the respiration response profiles as $E = 1/\Sigma pi^2$, [29, 30], where pi, is summed for all substrates and $p_i = r/\Sigma r_i$ (defined as the respiration response of each substrate (r_i) as a proportion of total respiration responses summed over all substrates (Σr_i)). Catabolic evenness is dimension-less, since it is a measure of relative variability in the catabolic functions.

Two year analysis was not significantly different so data were combined.The effects of herbicides on microbial catabolic evenness, richness and microbial biomass were examined by analysis of variance (ANOVA). Differences in catabolic evenness, richness and microbial biomass between treatments within each soil were then compared by ANOVA followed by HSD Tukey test.

RESULTS

In the first harvest, Anilofos+ethoxysulfuron treatment had the biggest and oxadiargyl the lowest size of biomass, respectively (Table 1). Though oxadiargyl treated soil had microbial biomass of 62% lower than Anilofos+ethoxysulfuron, but they did not showed significant difference ($p<0.05$). Different herbicides did not reduce the size of the microbial biomass at recommended herbicide application. In the second and the third harvest microbial biomass size had grown. Mean comparison did not show any meaningful differences among treatments in the second and third harvest (Table 1).

Table 1: Microbial biomass C (μg C g^{-1} soil) of different treatments (means ± standard error of mean ; r = 3)

Harvests								
Frist			Second			Third		
Treatment	Mean		Treatment	Mean		Treatment	Mean	
Anilofos+ ethoxysulfuron	588 ± 188	ns	Thiobencarb	832 ± 252	ns	Cinosulfuron	957 ± 76	ns
Thiobencarb	420 ±109	ns	Cinosulfuron	772 ±333	ns	Butachlor	856 ± 175	ns
Butachlor	336 ±101	ns	Check	762 ±243	ns	Anilofos+ ethoxysulfuron	756 ±168	ns
Cinosulfuron	336 ±92	ns	Anilofos+ ethoxysulfuron	756±286	ns	Check	740 ± 248	ns
Check	268 ±63	ns	Oxadiargyl	672 ± 289	ns	Thiobencarb	739 ± 241	ns
Oxadiargyl	218 ± 58	ns	Butachlor	621 ± 223	ns	Oxadiargyl	705 ± 254	ns

Tukey's studentized range (HSD) test was done for mean comparison. Within each harvest and treatment, values followed by the same lower case letter are not significantly different ($p \geq 0.05$), except Ns, where the effect of treatment was not significant.

Table 2: Microbial richness of different treatment (means ± standard error of mean ; r = 3) calculated by shanon index.

Harvests								
Frist			Second			Third		
Treatment	Mean		Treatment	Mean		Treatment	Mean	
Anilofos+ ethoxysulfuron	0.82 ± 0.14	ns	Cinosulfuron	0.78 ± 0.13	ns	Butachlor	0.79 ± 0.09	ns
Oxadiargyl	0.77 ±0.06	ns	Butachlor	0.64 ±0.19	ns	Oxadiargyl	0.77 ± 0.03	ns
Check	0.76 ±0.05	ns	Thiobencarb	0.56 ±0.16	ns	Check	0.7 ±0.07	ns
Thiobencarb	0.68 ±0.25	ns	Anilofos + ethoxysulfuron	0.52±0.13	ns	Anilofos + ethoxysulfuron	0.64 ± 0.13	ns
Cinosulfuron	0.67 ±0.05	ns	Check	0.47 ± 0.07	ns	Thiobencarb	0.63 ± 0.11	ns
Butachlor	0.57 ± 0.08	ns	Oxadiargyl	0.37 ± 0.17	ns	Cinosulfuron	0.42 ± 0.16	ns

Tukey's studentized range (HSD) test was done for mean comparison.Within each harvest and treatment, values followed by the same lower case letter are not significantly different ($p \geq 0.05$), except Ns, where the effect of treatment was not significant.

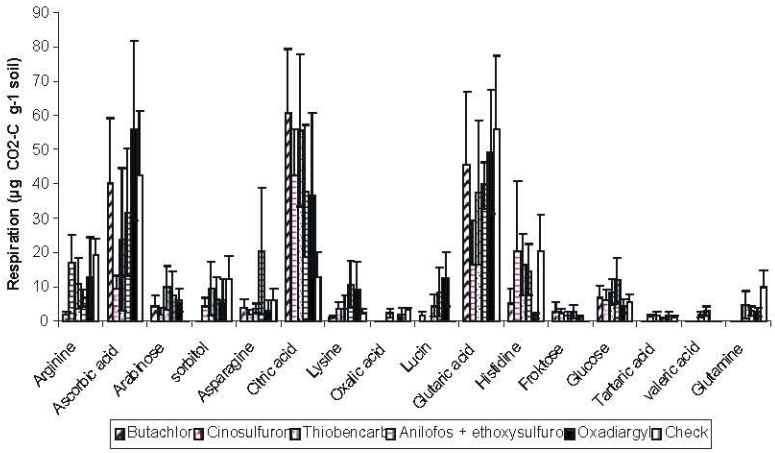

Fig. 1: Responses of respiration to different rice conventional herbicides

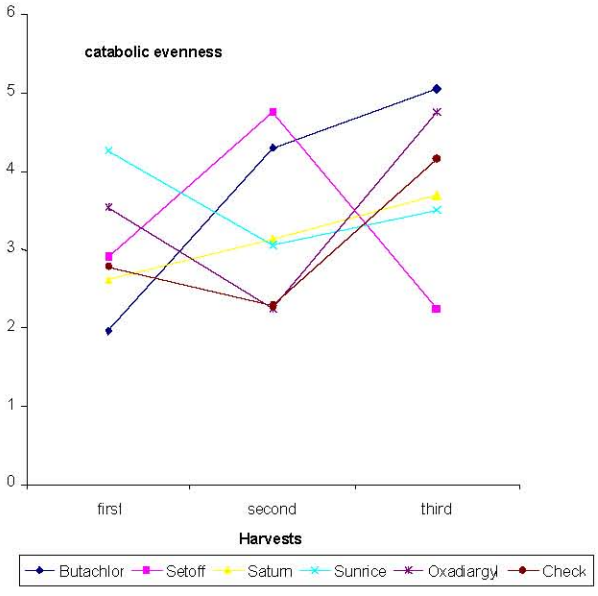

Fig. 2: Responses of microbial catabolic evenness to diferrent rice conventional herbicides

In first harvest, however Butachlors' Shanon index (0.5± 0.8) was 30% lower than Anilofos + ethoxysulfuron, it did not show significant difference ($p \leq 0.05$) (Table 2). The difference between higher and lower Shanon index in the second and the third harvests were 64% and 46% lower, respectively (Table 2).

In first harvest catabolic evenness for different treatments were between 2-4.2 units. Thiobencarb and Butachlor treated soil had the highest and lowest catabolic evenness, respectively. Though they had 2.2 unit difference but ANOVA did not show any significant difference ($P<0.05$) not only in this stage, but also in two other stages (data not shown). In the second and third harvest, the range of catabolic evenness did not change. Though the changes of catabolic evenness were inconsistent for different herbicide, there were not any differences between treatments at any harvest.

DISCUSSION

Rice conventional herbicides including Anilofos + ethoxysulfuron, Oxadiargyl, Thiobencarb, Cinosulfuron and Butachlor did not show any effect on soil microbial biomass. Furthermore, the biomass of communities was not greatly reduced by treatments, indicating that losses of population of organisms responsible for some catabolic functions did not occur as result of stress (different herbicides). Some workers reported that using herbicide at recommended dose do not have adverse effect on microbial biomass. Fraser *et al.* [31] conducted a field study to evaluate microbial populations and their activities under organic and conventional farm management and they observed that no significant differences were found in measured biological properties due to insecticide or herbicide at field application rates. Numerous studies on the effects of pesticides on microorganisms have not revealed any long-term harmful effects on numbers, composition and activities of microorganisms at least at normal field rates of application [5]. Data regarding the effects of Rimsulfuron treatments in laboratory trials showed that when applied at the filed rate, the microbial biomass C content was not significantly modified. When applied at 10 and 100 field rate the decreases in the microbial biomass C content became significant within the first 10 days [32]. Use of chemical fertilizers and pesticides at recommended application rates, however, has few direct long-term effects on microbial populations and their activity [33, 7].

Metabolic diversity of culturable bacteria also declined rapidly due to glypgosate [34]. Species richness (no. C compounds metabolized) at 0, 25, 50, 500 mM glyphosate was 39, 1, 0, 0 for Elkhorn (weak soil), 60, 34, 29, 0 for Whitmore (intermediate fertility); and 62, 28, 0, 0 for Feather fall (most productive), respectively. They also reported no difference between herbicide and control treatments in substrate-richness or diversity (Shanon Weaver Index) at three different soils with diverse productivity. In our experiment no difference were seen on diversity index indicating of limited differences between microbial communities.

Changes in catabolic evenness were attributable to changes in a range of substrate responses rather than changes in any one catabolic response or groups of catabolic responses. Examples of the typical changes in CRP's in response to the treatments are given in Figs. 2 and 3. In general, decreases in catabolic evenness were the result of a combination of decreases in responses to some substrates and slight increases to other substrates, whereas increases in catabolic evenness were the result of the reverse situation. Where there were large decreases in catabolic respiration, such as froktose and tartaric acid, most catabolic responses had decreased to less than that in the control soils.

In our work catabolic evenness did not change for treatments but for species evenness, community theory predicts that evenness should increase with stress as a result of reduced competition, smaller populations and reduced patchiness [30]. Catabolic evenness is a property of microbial communities that can change independently of changes in microbial biomass [21]. According to other works, only high level of stress can cause large changes in microbial community structure [35-37].

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