

EVALUATION OF TILLAGE AND WEED MANAGEMENT SYSTEMS ON RHIZOSPHERE MICROFLORA UNDER RICE-WHEAT CROPPING SYSTEM

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Abstract

A field study was conducted in an Inceptisol with rice-wheat cropping system during 2006 and 2007 to evaluate the effect of different tillage systems *vis-à-vis* different weed control practices on the population of fungi, total bacteria, actinomycetes, *Rhizobium* and *Azotobacter* in rhizosphere soil of rice and wheat. Four types of tillage systems and two methods of weed control practices were evaluated. Among weed control methods hand weeding and recommended herbicidal application (Butachlor as pre emergence and fenoxaprop ethyl + ethoxysulfuron as post emergence in *Kharif* and pendimethalin as pre emergence and Metsulfuron as post emergence in *rabi*) were compared. The study revealed that maximum microbial population in crop rhizosphere was found in conventional-conventional tillage system in both the seasons. However, conventional-zero and zero-conventional tillage systems were in second order in *Kharif* and *Rabi* season, respectively. Pre-emergence herbicides suppressed the microbial population between 0 and 10 days after sowing of crop, whereas application of post emergence herbicides did not show any significant inhibition in microbial population after their application. Among weed control practices, hand weeding was found most suitable for microbial population enhancement in soil followed by herbicide application. However, weedy check did not support the microbial population in soil in comparison to hand weeding practice.

Introduction

In India, rice-wheat cropping system is most commonly adopted cereal based cropping system. In Chhattisgarh state also most of the cropping systems comprise of rice which is grown as a major crop. There are several techniques adopted by the farmers for rice and wheat sowing as per their feasibility and resource availability. Line sowing is one of them which is not only economical but also save time. The most common problem associated with this system is the high density of weed flora which is the major threat and considered as a major pest. Hence it is essential to eradicate weeds which are the main competitor of plants for nutrients, sun light, moisture and space. Generally different types of tillage practices are adopted by the farmers to minimize the above problem. Herbicides are also applied to overcome this problem which is considered to be an efficient and economic method of weed control. Use of herbicides pre-emergent alone is a vital tool for effective and cost efficient weed control in direct seeded rice which encounters weed competition from the day of germination. However, limited application time window (0 - 5 DAS), a critical water regime and toxicity to main crop are associated challenges. In this scenario, post-emergence herbicides appear to offer alternate possibility (Mallikarjun *et al.* 2014). The physical, chemical and biological soil environment for zero till farming differs greatly from that of conventional tillage (Doran 1980), which is supposed to

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create a great impact on agricultural production (Ferreira *et al.* 2000). Similarly, the ultimate destination of herbicidal chemicals is the soil system where they come in contact with different microflora which are responsible for different biochemical transformations related to mineral nutrition to plants. Generally herbicides are not harmful when it is applied in recommended levels in soil (Selvamani and Sankaran 1993) but reports are there which show that herbicidal application has adverse effect on bacterial, fungal and actinomycetes population (Deshmukh *et al.* 2013). So the herbicide application definitely has some effect on microbial activities and microbial population as well. However, the effect of tillage systems and weed management practices (including herbicide application) on soil rhizosphere microflora under rice-wheat cropping systems was not adequately studied elsewhere. Therefore, the present study was conducted to evaluate the influence of different tillage and weed control systems on population of soil microflora and persistence of herbicides in soil in terms of microbial properties of crop rhizosphere.

Materials and Methods

Rice-wheat cropping system was rotated for three consecutive years (2005 - 2007) and in second (2006) and third year (2007) field studies were conducted to find out the effect of different tillage systems *vis-à-vis* different weed control practices on the population of three major microbial groups (bacteria, fungi and actinomycetes) and two crop beneficial groups (*Rhizobium* and *Azotobacter*) in rhizosphere soil. The experiments were conducted at research farm of Indira Gandhi Agricultural University, Raipur, Chhattisgarh located at 21.15° N latitude and 81.38° E longitude with an altitude of 298.56 m above the mean sea level. The experiment was conducted on *Kharif* rice (*Oryza sativa* L.) with test variety MTU-1010 and *Rabi* wheat (*Triticum aestivum*) with test variety GW-273. The soil was Inceptisol (Av. pH : 6.8, EC : 0.26 mili mhos/cm, organic carbon : 0.59%, available N: 205.50 kg/ha, available P: 12.80 kg/ha and available K : 348.00 kg/ha). Four types of tillage system were evaluated and put in main plots *viz.* (i) conventional-conventional, (ii) conventional-zero, (iii) zero-conventional and (iv) zero-zero tillage system. In conventional-conventional tillage system conventional tillage operation was done in *Kharif* as well as in *Rabi* season. Similarly in zero-zero tillage system zero tillage was adopted both the cropping season. However, in zero-conventional and conventional-zero system zero tillage followed by conventional tillage and conventional tillage followed by zero tillage operation was done, respectively in *kharif* and *rabi* season. Among weed control measures, performance of hand weeding and recommended herbicidal application was tested and compared with a weedy check and put in sub plots. As recommended herbicides for *Kharif* rice butachlor was sprayed in pre emergence and fenoxaprop-p-ethyl and ethoxysulfuron were sprayed in post emergence stage of the crop @ 1.5 kg, 56.25 gm and 15.00 gm/ha, respectively. Similarly in *Rabi* wheat pendimethalin and metsulfuron were sprayed at pre and post emergence stages of the crop @ 1.0 kg and 2 g/ha, respectively. The pre emergence and post emergence herbicides were applied at 3 and 20 days after sowing of the crop, respectively. The treatments were replicated thrice under split plot design. Soil from rhizosphere was collected from 7.5 - 15.0 cm, put in polythene bags and kept in refrigerator for microbial analysis. The soils from six rhizosphere zones were pooled together for the purpose of analysis. Soil sampling was done at 0, 5, 10, 20, 30 and 50 days after sowing of the crop and at harvest stage of the crops. The pooled soil samples were subjected to analysis for estimation of total bacteria, fungi, actinomycetes, *Rhizobium* and *Azotobacter* by serial dilution technique and pour plate method (Pramer and Schmidt 1965).

Enumeration of bacteria was done on asparagine mannitol agar media (Thornton 1922), fungi on Rose-Bengal agar (Martin 1950), actinomycetes on Kenknight and Munaier's medium, *Rhizobium* on YEMA and *Azotobacter* spp. on Jensen's nitrogen free medium (Jensen 1951). For

preparation of serial dilution, one gm of soil sample was suspended in nine ml of sterile water in a dilution tube (Tuladhar 1983) and shaken for 15 min. This constituted 10^{-1} concentration. Using a fresh sterile pipette took one ml of this suspension and nine ml sterile water was then added to get 10^{-2} dilution. The sequence was continued till a dilution of 10^{-7} reached. After preparation of different media, they were sterilized at 121°C for 15 min. One ml of desired dilution of freshly mixed suspension was transferred into the sterile Petri dish using sterile tip of micro-pipette. Subsequently, about 15 ml of partially cooled appropriate medium was poured into each plate and carefully swirl to thoroughly mix the contents. After the medium got solidified invert the plates and kept in an incubator at respective incubation temperature for different micro-organisms (28°C for fungi and 37°C for bacteria and actinomycetes). After specified period of growth (48 hrs for bacteria and actinomycetes and 96 hrs for fungi), colonies were counted and population was enumerated by using formula given by Schmidt and Caldwell (1967).

Number of bacteria/fungi/actinomycetes in 1 gm soil =

$$\frac{\text{No. of CFU} \times \text{dilution}}{\text{Dry weight of 1 gm moist soil} \times \text{aliquot taken}}$$

Results and Discussion

The results revealed that different tillage systems showed significant impact on microbial population in soil and this can be visualized at different time intervals except at initial (0 DAS) and at harvest stage where some parameters did not show variability with respect to different tillage systems. In this study, different tillage systems quantitatively altered the soil microflora (Tables 1 and 2). Maximum microbial population in rice rhizosphere was observed in conventional-conventional tillage system followed by conventional-zero tillage system in *Kharif* and zero-conventional tillage system in *Rabi* condition. Minimum population was observed in zero-zero tillage system. This growth variation under different tillage systems might be because of availability of different levels of oxygen under different tillage systems. Biswas and Mukherjee (1987) also reported that in normal arable soils, aerobic bacteria predominate. In the present study the variation in microbial population under different tillage systems may also be associated with the variation in organic matter distribution in soil profiles. Singh *et al.* (2007) also found higher microbial counts under conventional tillage system at lower soil depth (7.5 to 15 cm). They also reported that at lower layer i.e. more than 7.5 cm depth from soil surface, *Azotobacter* spp. counts were significantly higher under conventional tillage as compared to minimum tillage. They expressed that relatively higher availability of soil organic matter at lower soil profile (7.5 - 15.0 cm) under conventional tillage may be due to even distribution of crop residues and other nutrients throughout the plough zone. This may possibly accounted for observed higher counts of soil microflora at rice rhizosphere under conventional-conventional tillage than the zero-zero tillage system. Ferreira *et al.* (2000) also reported similar observations with regard to *Bradyrhizobium* spp. populations in soybean rhizosphere cultivated under zero tillage system. They found reduced soil microflora in lower layer (7.5 to 15 cm) under minimum tillage.

The effect of herbicides and hand weeding *vis-à-vis* weedy check on microbial population (Table 1) envisaged that in *Kharif* season application of pre emergence herbicide (butachlor) which sprayed at 3 days after sowing suppressed the fungal population from 43.0×10^3 (0 DAS) to 34.6×10^3 (10 DAS) which further increased to 49.0×10^3 at 20 days after sowing. Similarly the bacterial growth also inhibited from 0 to 10 DAS which was reflected by the population data of total bacteria recorded at 0 DAS (70.3×10^5) and 10 DAS (59.3×10^5), which further increased to 87.0×10^5 at 20 DAS. The population of actinomycetes and crop beneficial bacterial population (*Rhizobium* and *Azotobacter*) also showed the similar trend. Rajendran and Lourduraj (1999) also

Table 1. Effect of tillage and weed control practices on population dynamics of fungi ($\times 10^3$), Total bacteria ($\times 10^5$), actinomycetes ($\times 10^5$), *Rhizobium* ($\times 10^4$) and *Azotobacter* ($\times 10^3$) in rhizosphere soil (per gram dry soil) of rice (average data of two years).

Treatment	Days after sowing																								
	0					5					10					20									
	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo					
Tillage	47.9	79.8	68.3	56.9	50.0	46.5	80.2	68.7	57.1	50.8	50.7	83.8	70.7	58.3	51.9	72.2	118.1	99.7	84.3	72.1					
Con.-con.	40.9	68.4	57.7	47.8	45.1	41.0	69.5	57.6	48.0	45.6	44.4	72.9	59.3	48.9	46.3	63.4	104.1	85.9	72.3	63.3					
Con.-zer.	27.8	52.8	44.4	35.8	36.1	27.4	53.8	44.4	36.7	36.1	29.6	54.4	45.6	37.8	36.6	46.8	83.5	70.8	59.1	52.9					
Zer.-zer.	35.9	65.2	55.2	41.0	40.1	35.2	66.7	55.7	41.5	40.5	38.3	69.8	57.1	42.6	41.0	55.9	99.8	82.9	65.1	57.5					
Zer.-con.	0.7	0.6	0.7	0.6	0.7	0.5	0.7	0.5	0.6	0.7	0.6	0.7	0.6	0.7	0.8	1.7	1.0	1.3	0.7	0.8					
SEm \pm	1.9	1.8	2.0	1.6	2.0	1.4	1.8	1.4	1.7	1.9	1.6	2.0	1.7	1.9	2.1	5.1	3.0	3.4	1.8	2.1					
CD (0.05)																									
Weed management																									
Hand weeding	36.4	65.9	55.7	44.1	41.8	37.4	71.0	59.4	47.9	45.0	44.7	78.3	63.9	51.9	47.9	66.0	111.7	92.4	76.9	67.3					
Re. herb. appl.	43.0	70.3	59.7	48.6	45.7	36.7	63.5	53.9	42.2	40.7	34.6	59.3	50.1	38.1	37.1	49.0	87.0	74.4	58.7	51.6					
Weedy check	35.0	63.5	53.9	43.3	41.0	37.5	68.1	56.6	47.3	44.1	43.0	73.1	60.5	50.6	46.7	63.7	105.5	87.7	75.0	65.6					
SEm \pm	0.5	0.7	0.6	0.5	0.5	0.3	0.8	1.0	0.4	0.4	0.3	0.7	0.8	0.8	0.5	1.2	1.5	1.3	0.8	0.8					
CD (0.05)	1.5	NS	NS	NS	NS	1.4	2.4	2.9	1.4	1.3	1.1	2.0	2.1	2.0	1.5	3.5	4.2	3.8	2.1	2.1					
Days after sowing																									
Treatment	30															50					At harvest				
	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo					
	Tillage	95.8	173.1	144.8	135.2	120.0	114.6	215.3	176.8	169.2	152.5	68.0	90.7	78.0	70.9	63.7									
Con.-con.	81.0	152.7	127.2	118.5	106.3	96.6	190.3	158.8	149.3	135.6	65.4	83.4	76.2	67.6	61.4										
Con.-zer.	62.6	130.1	109.4	103.6	93.7	77.4	166.3	137.2	131.7	121.0	61.1	78.0	73.8	64.6	55.7										
Zer.-zer.	73.3	147.9	122.8	110.8	99.3	88.7	184.1	152.4	140.1	127.8	64.4	82.1	75.8	61.5	58.7										
Zer.-con.	1.2	2.6	1.5	1.6	1.1	1.2	1.1	2.0	1.6	1.3	1.2	0.6	0.9	1.1	0.7										
SEm \pm	3.5	8.2	4.1	4.7	3.3	3.4	3.2	5.4	4.7	3.5	NS	1.6	NS	3.4	NS										
CD (0.05)																									
Weed management																									
Hand weeding	92.9	170.2	141.1	131.1	117.1	111.6	207.7	172.1	161.3	146.5	67.9	86.2	78.4	68.5	62.9										
Re. herb. appl.	52.3	121.4	101.7	91.7	82.5	63.9	161.8	131.5	124.0	113.3	60.3	79.5	72.7	61.6	55.4										
Weedy check	89.3	161.2	135.3	128.3	114.9	107.6	197.5	165.3	157.4	142.9	66.1	84.9	76.8	68.4	61.5										
SEm \pm	0.9	1.8	1.3	1.1	1.3	1.5	1.1	1.4	1.6	1.6	1.2	0.8	0.9	0.9	0.8										
CD (0.05)	2.8	5.1	3.8	3.1	3.9	4.3	3.2	3.9	4.8	4.7	NS	2.6	NS	NS	2.7										

Table 2. Effect of tillage and weed control practices on population dynamics of fungi ($\times 10^3$), Total bacteria ($\times 10^5$), actinomycetes ($\times 10^5$), *Rhizobium* ($\times 10^4$) and *Azotobacter* ($\times 10^3$) in rhizosphere soil (per gram dry soil) of wheat (Average data of two years).

Treatment	Days after sowing																			
	0					5					10					20				
	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo
Tillage																				
Con.-con.	62.2	80.5	75.7	65.0	57.5	61.7	78.9	71.9	64.9	57.3	64.7	82.9	73.9	66.1	57.9	82.9	116.0	92.5	88.0	75.8
Con.-zer.	51.0	69.6	66.2	55.0	51.3	50.0	67.3	63.3	52.4	49.3	50.5	69.7	63.6	52.9	49.1	62.6	94.9	79.8	69.8	62.9
Zer.-zer.	43.0	57.3	51.0	42.1	42.7	42.7	56.6	50.2	41.7	42.4	43.7	57.8	51.9	42.3	42.9	56.2	73.9	66.5	55.2	55.5
Zer.-con.	52.4	70.0	62.1	54.7	49.6	52.6	71.6	65.4	57.0	52.0	55.1	73.6	67.7	57.4	52.4	71.8	101.1	84.4	75.1	67.4
SEM \pm	0.8	0.7	0.7	0.7	0.8	1.0	0.8	1.0	1.1	1.0	1.1	1.1	1.0	1.5	1.1	0.8	1.2	1.2	1.1	1.0
CD (0.05)	2.5	2.2	2.3	2.0	2.4	2.8	2.5	3.1	3.2	2.9	3.9	3.2	2.8	4.2	3.2	2.5	3.8	3.6	3.2	2.8
Weed management																				
Hand weeding	49.9	66.7	60.2	53.6	49.4	52.0	71.1	63.3	57.8	52.8	57.8	77.7	67.6	61.9	56.0	77.5	109.9	85.8	83.7	73.0
Re. herb. appl.	57.6	74.0	66.4	55.1	53.1	52.3	63.9	56.9	48.9	46.4	46.2	57.8	52.9	42.9	41.5	53.5	72.1	65.9	53.8	53.2
Weedy check	48.9	67.3	64.7	51.8	48.3	50.9	70.9	67.9	55.3	51.6	56.5	77.5	72.3	59.2	54.2	74.1	107.4	90.8	78.7	70.0
SEM \pm	0.8	0.7	1.4	0.9	0.6	0.6	1.0	1.0	0.7	0.8	0.8	0.8	0.8	1.2	1.1	1.1	1.2	0.8	0.7	1.2
CD (0.05)	NS	2.1	NS	NS	NS	NS	2.7	2.6	2.2	2.5	2.4	2.3	3.4	2.9	3.2	3.8	2.5	2.1	3.4	3.2
Treatment	Days after sowing															At harvest				
	30					50					50					At harvest				
	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo	Fun	TB	Act	Rhi	Azo
Tillage																				
Con.-con.	103.4	165.0	107.5	125.9	119.4	115.2	194.2	132.0	144.6	140.6	144.6	140.6	140.6	140.6	140.6	55.5	78.3	69.4	63.6	52.6
Con.-zer.	78.1	119.1	92.9	104.8	93.4	87.0	158.8	113.2	123.1	108.3	123.1	108.3	108.3	108.3	108.3	46.1	72.4	65.6	57.7	44.7
Zer.-zer.	69.1	112.3	78.6	88.6	84.1	76.9	132.6	96.2	104.3	98.9	104.3	98.9	98.9	98.9	98.9	44.4	70.4	64.1	56.2	43.0
Zer.-con.	88.7	145.8	97.8	112.2	99.4	98.4	170.2	119.3	132.5	115.4	119.3	132.5	115.4	115.4	115.4	47.3	73.2	66.4	61.0	48.3
SEM \pm	0.8	0.8	1.5	1.5	1.8	1.5	1.4	1.7	2.0	1.5	1.7	2.0	1.5	0.6	0.6	0.6	1.0	0.7	0.8	0.7
CD (0.05)	2.4	2.5	4.2	4.1	4.6	4.2	3.8	4.3	5.2	4.6	4.3	5.2	4.6	NS	NS	NS	NS	NS	NS	2.0
Weed management																				
Hand weeding	101.5	159.1	112.4	133.0	116.7	112.6	183.3	137.3	146.5	130.4	146.5	130.4	130.4	130.4	130.4	50.8	75.9	67.6	61.9	49.5
Re. herb. appl.	55.2	105.8	52.4	65.8	68.1	60.9	132.8	63.8	93.9	91.0	63.8	93.9	91.0	44.2	44.2	69.1	61.7	55.3	43.0	43.0
Weedy check	97.8	141.7	118.0	124.7	112.4	109.6	175.8	144.4	140.1	126.9	140.1	126.9	126.9	50.1	50.1	75.8	69.8	61.8	48.9	48.9
SEM \pm	1.1	1.0	1.0	1.3	1.2	1.2	1.1	1.2	1.1	1.2	0.8	2.5	1.2	0.5	0.5	1.0	0.5	0.5	0.8	0.7
CD (0.05)	3.2	3.1	2.8	3.6	3.4	3.4	2.9	2.5	6.8	3.6	2.5	6.8	3.6	NS	NS	NS	NS	1.4	NS	NS

reported that population of bacteria, fungi and actinomycetes were affected with butachlor application in rice and these adverse effects gradually reduced with passage of time. Nongmaithem and Pal (2012) also expressed similar views and mentioned that decrease in the population of microflora might be due to toxic effect of the applied chemical herbicides. It may be noted that the effects of herbicides on the soil microflora are normally most severe immediately after their application. The application of post emergence herbicides (fenoxaprop- p-ethyl and ethoxy sulfuron) which were sprayed at 20 days after crop emergence did not suppress the growth of micro-organisms under study which was visualized by their respective population at 20, 30 and at 50 DAS in rhizosphere soil. However, the rate of growth of microbial populations from 20 to 30 DAS was comparatively slower than the growth from 30 to 50 DAS. The fungal population was recorded 49.0×10^3 at 20 DAS which further increased to 52.3 (30 DAS) and further to 63.9×10^3 at 50 DAS. Similarly the bacterial population as a whole increased from 87.0×10^5 (20 DAS) to 121.4 (30 DAS) and further to 161.8×10^5 . The population of actinomycetes and crop beneficial bacterial population (*Rhizobium* and *Azotobacter*) also showed the similar trend. The findings revealed that the persistence of post emergence herbicides which was taken under study (fenoxaprop-p-ethyl and ethoxy sulfuron) was very less in comparison to pre-emergence herbicide (butachlor) whose toxic effect to microorganisms found in soil was about 7 days. Roger *et al.* (1994) also concluded that faster degradation of herbicides in tropical rice fields includes reducing conditions, favourable temperature, pH and the presence of flood water.

In *Rabi* season also application of pre-emergence herbicide pendimethalin and metsulfuron suppressed the fungal population from 57.6×10^3 (0 DAS) to 46.2×10^3 (10 DAS) which further increased to 53.5×10^3 at 20 days after sowing. Similarly the bacterial growth also inhibited from 74.0×10^5 (0 DAS) to 57.8×10^5 (10 DAS), which further increased to 72.1×10^5 at 20 DAS. The population of actinomycetes and crop beneficial bacterial population (*Rhizobium* and *Azotobacter*) also showed the similar trend in case of wheat. Stimulation in the bacterial population could be attributed to the increment of resistant organisms which utilized the herbicides as a nutrient source (Wardle and Parkinson 1990). The application of post emergence herbicide metsulfuron did not suppress the growth of microorganisms which was reflected in population dynamics study. However, the rate of growth of microbial populations from 20 to 30 DAS was comparatively slower than the growth from 30 to 50 DAS. The fungal population was recorded 53.5×10^3 at 20 DAS which further increased to 55.2 (30 DAS) and further to 60.9×10^3 at 50 DAS. Similarly the bacterial population as a whole increased from 72.1×10^5 (20 DAS) to 105.8 (30 DAS) and further to 132.8×10^5 . The population of actinomycetes and crop beneficial bacterial population (*Rhizobium* and *Azotobacter*) also showed the similar trend.

One of the reasons of less harmful effect of post emergence herbicides on microorganisms may be more foliage of crops at 22 DAS (at the time of herbicides application) which covers the soil surface resulting less dropping and absorption of applied herbicides in the soil. On the contrary the dropping and absorption of pre-emergence herbicides in soil is more, which is because of necked soil surface at the time of application of pre-emergence herbicide.

Among weed control practices, hand weeding was found most suitable for microbial population enhancement in soil followed by herbicide application. However, weedy check did not support the microbial population in soil in comparison to hand weeding practice. Among different kind of microorganisms, the reduction of bacterial population is higher in comparison to actinomycetes and fungi in case of pre-emergence herbicidal application. Nalayini and Sankaran (1992) also revealed that fungi showed resistance to the herbicides. Similarly, rhizobial population was affected more than *Azotobacter* by pre-emergence herbicide application. In this study maximum microbial population was observed at 50 DAS and thereafter declination of the population was noticed up to harvest of the crop.

It is concluded from the above study that conventional-conventional tillage system facilitated the growth of microorganisms in crop rhizosphere in both *Kharif* and *Rabi* seasons. Among weed control practices, hand weeding was found most suitable for microbial population enhancement in soil followed by herbicide application. Pre-emergence herbicides suppressed the microbial population between 0 and 10 days after sowing of crop (DAS), whereas application of post emergence herbicides did not show any significant inhibition in microbial population after their application.

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