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Full Length Article

Response of Soil Microflora and Earthworms to Pyrazosulfuron Ehtyl, A New Generation Herbicide In Transplanted Rice In The Entisols Of Vellayani, South Kerala

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ABSTRACT
Indiscriminate and continuous application of herbicides in an intensive cropping system may adversely affect the soil ecosystem including soil micro and macro organisms. Pyrazosulfuron ethyl (PSE) is a low dose high efficacy herbicide coming under the group of sulfonyl ureas which is effective for controlling a wide range of weeds in low land rice. Field experiments were conducted for two consecutive seasons ie., second and third crop seasons at the Instructional Farm, College of Agriculture, Vellayani to study the effect of PSE on soil microflora and earthworms in transplanted rice in the entisols of Vellayani, South Kerala. The experiment was laid out in randomized block design which consisted of eight treatments with three replications. The treatments included four different levels of pyrazosulfuron ethyl (15, 20, 25 and 30 g ai ha^{-1}), butachlor (1.5 kg ai ha^{-1}), weed free check, unweeded check and hand weeding twice (at 20 and 40 days after transplanting). The control plots (weed free check and unweeded check) and farmers practice (hand weeding twice) recorded higher fungal and bacterial population and were significantly different from all other herbicide treatments. Herbicides significantly caused an inhibitory effect on the growth of fungi and bacteria in the initial stages; however their population recovered at 30 days. The population of actinomycetes and earthworm was not affected by the herbicide or its concentration. The restoration of microbial and earthworm population shows that the delicate biological balance of the soil is very little affected by application of PSE. This emphasizes the safety of chemical to soil biological balance.

Key words: *rice, herbicides, pyrazosulfuron ethyl, soil micro organisms, earthworms*
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INTRODUCTION

Rice is the most important and extensively grown crop in India and it serves as staple food for millions of people. One of the major constraints in rice production is competition from weeds. Proper weed management is essential to realize the potential yield of high yielding rice varieties. Due to the exorbitant wage rate combined with low efficiency and non availability of labour during the peak periods in Kerala State, where rice is the staple diet of the people, hand weeding which is the most common weed control method has become a burden for the rice farmers. Hence the chemical weed control is more effective and economical than mechanical weed control. The herbicides applied at their recommended doses have only temporary effect on micro organisms. The soil micro organisms which include bacteria, fungi, actinomycetes and algae play a crucial role in carbon flow, nutrient cycling and litter decomposition which in turn affect the soil fertility and plant growth (Chauhan *et al.,*2006; Tripathi *et al.,* 2006 and Pandey *et al.,* 2007). A healthy population of soil micro organisms can stabilize the ecological system in in soil (Chauhan *et al.,*2006). The soil micro organisms are very resilient due to its large populations, short reproductive cycles and greater adaptability to environment. Any change in their population and activity may affect nutrient cycling as well as availability of nutrients which indirectly affect productivity and other soil functions(Wang *et al.,*2008). It is also reported that one of the possible causes of productivity decline in rice cropping system is the change in soil microflora (Reichardt *et al.*, 1998).The indiscriminate, widespread and continuous application of herbicides adversely affect the soil ecosystem, *viz.* microflora and macroflora such as plant pathogens, antagonists, nematodes, insects and earthworms.
Sulfonyl urea, a new group of herbicides which are highly effective at very low rate of application, is gaining popularity among the farming community. This group comprises the most widely used herbicides in the present day agriculture (Rao, 2000). With these herbicides there is a possibility of reducing the dose of the chemical by 100 to 1000 times over traditional herbicides (Brown, 1990) making them

environmentally safe. Hence this group of herbicides is also called as low dose high efficacy (LDHE) herbicides. Among the various LDHE herbicides pyrazosulfuron ethyl (PSE) is very effective against grasses, sedges and broad leaved weeds in rice (Moorthy, 2002). The bioefficacy of different sulfonyl urea herbicides in rice have been studied by various scientists. A little research work has been done on the effect of pyrazosulfuron ethyl on soil micro organisms and earth worms in transplanted rice. Hence an attempt was done to study the effect of PSE on soil microflora and earthworms in transplanted rice in the entisols of Vellayani, South Kerala.

MATERIALS AND METHODS

The field experiments were conducted during the second(September to December) and third crop season (January to April) at the Instructional Farm, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala,India to study the effect of PSE on soil microflora and earthworms in transplanted rice. The soil was medium in available nitrogen (277.65) and high in available phosphorus (27.03), available potassium (331.52 kg ha^{-1}) and organic carbon (1.19). The experiment consists of 8 treatments with three replications. The plot size was 6X4 m^2. The variety Aiswarya (medium duration) was transplanted at a spacing of 20x10 cm. The treatments included pyrazosulfuron ethyl (PSE)@ 15 gai ha^{-1}(T_1), 20 gai ha^{-1}(T_2), 25 gai ha^{-1}(T_3) and 30 gai ha^{-1}(T_4),butachlor @1.5kgai/ha(T_5), weed free check(T_6), unweeded check(T_7) and farmers' practice ie, hand weeding twice at 20 and 40 DAT(T_8). Herbicide PSE was applied as early post emergence (at 10 DAT) and butachlor as pre emergence (at 6 DAT) and hand weeding was done as per the treatment. The formulations used were Saathi 20 WP and butachlor 50 EC. All the herbicides were applied as spray using 500 L of water per hectare. All the recommended package of practices other than weed control was adopted to raise the experimental crop. Estimation of earthworms was carried out before herbicide spraying and after the harvest during the second and third crop season.

ENUMERATION OF FUNGI, BACTERIA AND ACTINOMYCETES IN SOIL

During the second and third crop seasons, for the enumeration of soil micro organisms (fungi, bacteria and actinomycetes) soil samples were taken at 0-15 cm depth just before spraying, at 3, 6, 15 and 30 DAS and at harvest using core sampler. Five samples collected randomly from each plot were mixed thoroughly to form a composite sample and used for the enumeration of soil micro organisms. The total count of fungi, bacteria and actinomycetes was assessed by serial dilution plate technique (Johnson and Curl, 1972) using soil extract agar, Martin's Rose Bengal agar and KenKnight's Agar respectively. Observations were taken for the colonies after 24 hours in the case of bacteria, 72 hours for fungi and 154 hours for actinomycetes in colony forming units per gram dry weight of soil.

QUANTITATIVE ESTIMATION OF EARTHWORMS

Five samples from each plot were collected and earthworm population was estimated. One metre square wooden frame was used for marking the sampling area. Digging was done up to about 10 cm depth (Bano and Kale, 1991). The soil lumps were broken and soil passed through the fingers to sort out the worms. The smaller worms were collected by passing through a sieve of 3-4 mm size. The worms were then counted.

The data generated were subjected to analysis of variance (ANOVA) after transformation ($\sqrt{x+1}$ transformation)as applied to randomized block design (Panse and Sukhatme, 1985). Whenever the results were significant, the critical difference was worked out at five per cent probability.

RESULTS AND DISCUSSION

Soil Fungal Population

A perusal of the data on fungal population furnished in table 1 revealed that there was significant difference among the treatments, at 3 days after spraying (DAS) and 6 DAS, during both seasons.

During the second crop season, initially there was a reduction in fungal growth at 3 DAS, but gradually fungus regained its growth and population was enhanced. Unweeded check recorded the highest number of fungi at 3 DAS which was statistically on par with hand weeding twice and weed free check and was significantly different from all other treatments. The lowest count was recorded by PSE @ 15 g ai ha^{-1} which was comparable with PSE @ 30, 25, 20 and butachlor @ 1.5 kg ai ha^{-1}. At 6 DAS also HWT, unweeded check and weed free check registered higher fungal count and were statistically on par. They varied significantly from all other treatments. PSE at different doses and butachlor @ 1.5 kg ai ha^{-1} recorded lower number of fungal colonies. From 15 DAS onwards the effect of treatments was non-significant. During the third crop season, fungal count decreased upto 3 DAS in all treatments followed by a gradual increase till the end of the crop growth. Here also the same treatments those were effective during the second crop season, recorded higher number of fungal colonies. The lowest count was registered by PSE @ 20 g ai ha^{-1} which was statistically on par with PSE @ 15, 25 and 30 g ai ha^{-1} and standard rice herbicide butachlor @ 1.5 kg ha^{-1}. At 6 DAS also all treatments including PSE and butachlor recorded lower fungal count, which was significantly different from all other treatments. However, the impact of herbicides on fungal count was not significant from 15 DAS upto harvest though population showed on increasing trend. Herbicides caused an inhibitory effect on the growth of fungi in the initial stages, however fungal population increased after 30 days (Deshmukh and Khande, 1977). The initial inhibition of soil fungi in the PSE and butachlor treated plot might be due to the competitive influence of various microorganisms on the population as well as toxic effect of the herbicides applied. In this experiment also, the effect of herbicides towards soil fungi decreased with time and within 30 days after spraying the fungi reached its original population which is due to microbial adaptation to these herbicides or due to their degradation. It can also be due to the microbial multiplication on increased supply of nutrients available in the form of micro organisms killed by the herbicides (Latha and Gopal, 2010; Vandana *et al.,* 2012).

Table 1 Effect of pyrazosulfuron ethyl on the population of soil fungi

Treatments	Population of soil fungi (x10 ^{4} cfu g^{-1} soil)											
	Second crop season						Third crop season					
	Before spraying	3 DAS	6 DAS	15 DAS	30 DAS	Harvest	Before spraying	3 DAS	6 DAS	15 DAS	30 DAS	Harvest
T_1	16.33	2.96 (1.99)	5.56 (2.56)	10.59 (3.40)	15.56 (4.07)	18.23 (4.38)	29.67	7.24 (2.87)	10.63 (3.41)	24.32 (5.03)	28.89 (5.47)	30.32 (5.59)
T_2	16.00	3.97 (2.23)	6.28 (2.69)	12.64 (3.69)	16.28 (4.16)	17.29 (4.28)	28.67	7.20 (2.83)	12.12 (3.62)	28.63 (5.44)	28.22 (5.41)	32.62 (5.78)
T_3	15.70	3.52 (2.12)	7.92 (2.99)	9.94 (3.31)	14.92 (3.99)	16.54 (4.19)	27.00	7.29 (2.88)	12.95 (3.74)	25.82 (5.18)	27.19 (5.31)	30.82 (5.64)
T_4	15.83	3.32 (2.08)	6.61 (2.76)	8.98 (3.16)	16.61 (4.19)	14.96 (3.99)	28.33	9.60 (3.26)	10.91 (3.45)	26.93 (5.28)	27.21 (5.31)	33.92 (5.91)
T_5	16.67	5.27 (2.50)	5.66 (2.58)	9.94 (3.11)	15.66 (4.08)	15.31 (4.04)	29.67	11.27 (3.50)	12.25 (3.64)	23.83 (4.98)	28.63 (5.44)	33.83 (5.91)
T_6	15.86	12.92 (3.73)	13.38 (3.79)	13.62 (3.82)	14.95 (3.99)	18.65 (4.43)	29.33	20.97 (4.69)	19.95 (4.58)	26.26 (5.22)	29.30 (5.50)	31.82 (5.73)
T_7	16.30	16.28 (4.16)	14.36 (3.92)	12.22 (3.77)	16.31 (4.16)	17.94 (4.35)	29.33	21.30 (4.72)	26.95 (5.29)	25.98 (5.19)	28.94 (5.47)	32.41 (5.78)
T_8	17.33	14.56 (3.94)	17.22 (4.27)	11.30 (3.51)	17.58 (4.31)	17.28 (4.28)	29.67	23.27 (4.93)	28.93 (5.47)	26.13 (5.21)	27.63 (5.35)	34.73 (5.98)
SE	*	0.179	0.169	-	-	-	*	0.166	0.202	-	-	-
CD		0.536	0.506	-	-	-		0.497	0.606	-	-	-

*Mean values, Transformed values (($\sqrt{x+1}$ transformation) are given in parenthesis

Soil bacterial Population

Bacterial population decreased upto 6 DAS in all PSE treated plots and then increased (table 2). In all treatments, the bacterial population reached almost original level at harvest during both seasons. During second crop season, at 3 DAS the highest count was registered by T_7 which was on par with T_8, T_6, T_2 and T_1 (HWT, weed free check, PSE @ 20 and 15 g ai ha^{-1} respectively) while the lowest bacterial count was recorded by PSE @ 30 g ai ha^{-1} and was on par with T_3 and T_5 (PSE @ 25 g ai ha^{-1} and butachlor @ 1.5 kg ai ha^{-1} respectively). At 6 DAS, T_8 recorded the highest bacterial count, which was on par with T_7 and T_6 and was significantly different from all other treatments except T_5. PSE @ 30 g ai ha^{-1} recorded the lowest count and was on par with T_3, T_2 and T_1. During the third crop season, at 3 DAS, unweeded check recorded the highest bacterial population that was statistically comparable with HWT and weed free check. This was followed by T_1 (PSE @ 15 g ai ha^{-1}) and this treatment was on par with HWT (T_8) and weed free check (T_6). The lowest count was given by T_3 (PSE @ 25 g ai ha^{-1}), which was on par with T_4 and T_5 (PSE @ 30 g ai ha^{-1} and butachlor @ 1.5 kg ai ha^{-1}) respectively. At 6 DAS, T_8 (HWT) with higher bacterial count was on par with T_7 (unweeded check) and T_6 (WFC) and the above treatments were significantly superior to all treatments involving PSE during the third crop season. However the lowest count was recorded by PSE @ 25 g ai ha^{-1} which was statistically comparable with T_4, T_1 and T_2. From 15 DAS onwards the bacterial count showed an increasing trend. The control plots recorded the highest bacterial population and were significantly different from all other herbicide treatments. The herbicides can cause an inhibitory effect on bacterial population however recovered within 30 days (Mandal *et al.*, 1987; Latha and Gopal,2010). The decrease in the bacterial population at initial stages of application of herbicides might be due to the competitive influence and toxic effects of herbicides. Gradually the population of soil micro organisms increased and regained its original position at 15-30 days. This increased count might be due to the release of carbon from degraded chemicals which might be beneficial to bacteria as nutrient and energy source that leads to its multiplication. Sapundjieva *et al.*,(2008), Sebiomo *et al.*,(2011) and Vandana *et al.*,(2012) reported similar findings.

Table 2 Effect of pyrazosulfuron ethyl on the population of soil bacteria

Treatments	Population of soil bacteria (x10 ^{4} cfu g^{-1} soil)											
	Second crop season						Third crop season					
	Before spraying	3 DAS	6 DAS	15 DAS	30 DAS	Harvest	Before spraying	3 DAS	6 DAS	15 DAS	30 DAS	Harvest
T_1	35.67	21.57 (4.75)	17.61 (4.31)	28.93 (5.47)	33.56 (5..96)	38.24 (6.41)	38.67	20.90 (4.68)	16.96 (4.24)	24.94 (5.09)	34.93 (5.38)	38.22 (6.26)
T_2	36..33	30.09 (5.58)	14.57 (3.94)	23.82 (4.98)	30.21 (5.59)	34.31 (5.94)	39.33	16.93 (4.23)	17.31 (4.28)	23.90 (4.99)	39.28 (5.50)	40.21 (6.42)
T_3	36.00.	29..65 (5.56)	14.31 (3.91)	24.30 (5.03)	35.62 (6.05)	38.91 (6.47)	38.33	11.64 (3.55)	15.31 (4.04)	22.94 (4.89)	37.62 (6.53)	39.63 (6.37)
T_4	35.69	19.97 (4.58)	11.95 (3.69)	24.61 (5.06)	33.29 (5.49)	36.25 (6.10)	39.00	11.95 (3.59)	16.49 (4.18)	24.32 (5.03)	35.21 (6.02)	37.98 (6.240
T_5	36.67	22.58 (4.86)	26.23 (5.22)	25.27 (5.13)	32.30 (5.77)	38.58 (6.29)	38.67	13.64 (3.83)	23.27 (4.93)	23.95 (4.99)	36.97 (5.47)	36.96 (6.16)
T_6	35.66	33.22 (5.85)	32.23 (5.76)	30.41 (5.60)	32.95 (6.39)	37.27 (6.19)	39.67	26.58 (5.25)	27.31 (5.32)	31.12 (5.67)	38.61 (5.44)	40.92 (6.47)
T_7	36.67	37.12 (6.17)	33.36 (5.86)	30.07 (5.57)	33.57 (5.87)	39.27 (6.35)	40.33	27.59 (5.35)	27.90 (5.38)	34.25 (5.94)	35.30 (6.03)	37.60 (6.21)
T_8	36.33	36.49 (6.12)	37.72 (6.22)	32.30 (5.77)	34.21 (5.93)	39.10 (6.33)	38.00	29.49 (5.15)	29.26 (5.50)	31.73 (5.72)	35.96 (6.08)	36.59 (6.13)
SE	*	0.247	0.251	-	-	-	*	0.195	0.180	0.191	-	-
CD		0.739	0.752	-	-	-		0.584	0.541	0.574	-	-

*Mean values, Transformed values (($\sqrt{x+1}$ transformation) are given in parenthesis

Table 3 Effect of pyrazosulfuron ethyl on the population of soil actinomycetes

Treatments	Population of soil actinomycetes (x10 ^{6} cfu g^{-1} soil)											
	Second crop season						Third crop season					
	Before spraying	3 DAS	6 DAS	15 DAS	30 DAS	Harvest	Before spraying	3 DAS	6 DAS	15 DAS	30 DAS	Harvest
T_1	3.33	1.94 (1.72)	2.32 (1.82)	1.91 (1.73)	2.32 (1.82)	2.96 (1.99)	3.67	2.96 (1.99)	2.61 (1.90)	1.49 (1.58)	2.65 (1.91)	4.97 (2.44)
T_2	2.67	2.32 (1.82)	2.61 (1.90)	1.49 (1.58)	2.65 (1.91)	2.61 (1.90)	3.33	1.49 (1.58)	0.91 (1.38)	1.49 (1.58)	2.00 (1.73)	3.65 (2.16)
T_3	3.67	1.94 (1.72)	2.05 (1.75)	1.64 (1.63)	2.64 (1.63)	2.32 (1.82)	3.33	1.21 (1.49)	1.94 (1.72)	2.22 (1.79)	2.32 (1.82)	3.58 (2.14)
T_4	3.00	1.21 (1.49)	2.61 (1.90)	1.55 (1.60)	1.31 (1.52)	2.65 (1.91(	3.67	1.49 (1.58)	1.59 (1.61)	1.94 (1.72)	3.28 (2.07)	2.96 (1.99)
T_5	3.33	1.17 (1.47)	1.98 (1.73)	1.44 (1.58)	2.30 (1.82)	2.96 (1.99)	3.33	2.65 (1.91)	1.74 (1.66)	1.49 (1.58)	3.97 (2.23)	3.65 (2.16)
T_6	2.67	2.96 (1.99)	3.25 (2.06)	2.32 (1.82)	2.61 (1.90)	3.97 (2.23)	4.00	3.97 (2.23)	3.32 (2.08)	2.96 (1.99)	3.32 (2.08)	3.97 (2.22)
T_7	3.33	3.97 (2.23)	3.97 (2.23)	3.97 (2.23)	2.96 (1.99)	4.26 (2.29)	2.67	4.26 (2.29)	3.58 (2.14)	3.28 (2.07)	3.65 (2.16)	5.27 (2.50)
T_8	2.67	3.86 (2.20)	4.26 (2.290	2.32 (1.82)	2.96 (1.99)	4.32 (2.31)	3.67	3.32 (2.08)	4.63 (2.37)	3.32 (2.08)	4.32 (2.31)	5.32 (2.51)
SE	*	-	-	-	-	-	*	-	-	-	-	-
CD		-	-	-	-	-		-	-	-	-	-

*Mean values, Transformed values (($\sqrt{x+1}$ transformation) are given in parenthesis

Effect on Soil Actinomycetes

The actinomycetes population was not affected by the herbicide or its concentration (table 3). Their constant population in soil is extremely useful as these microbes play a major role in the biodegradation of pesticides in soil. According to Beyer *et al*. (1988), the soil actinomycetes *Streptomyces griseolus* rapidly metabolises chlorsulfuron. Generally these micro organisms are very important in the degradation of herbicides (Rao, 2000).

EFFECT OF HERBICIDES ON EARTHWORM IN SOIL

Before the start of experiment, the composite soil sample recorded an earthworms population of 14 m^{-2} of soil. After the harvest of the second crop season also earthworm population could be observed in the experimental plots and no significant reduction in the population was noticed compared to the initial one. However after the harvest of the third crop, irrespective of the treatment, earthworm could not be observed in the experimental field while the moist soil in the premises of the nearby canal contained earthworms (Table 4). The absence of earthworms in the experimental field could probably be due to drying up of the soil due to higher temperature and low rain fall experienced during the summer season. The total rainfall received during this season was only 1.77 mm compared 314.46 mm in the second crop season. Similar observation on drastic reduction in earthworm count due to reduced moisture content and higher temperature was reported by Jayasree (2005). Hallett *et al*. (1992) found that moisture influenced the growth and reproduction of earthworm and most favourable moisture content is 80 per cent. According to Mosleh *et al*. (2003), isoproturon @ 1.4 g kg^{-1} soil had no lethal effect on earthworms. Similar observation on the non lethal effect of atrazine and metolachlor on earthworm population at the recommended dose was made by Farenhorst *et al*. (2003).

Table 4 Effect of pyrazosulfuron ethyl on earthworm population after harvest of the second crop

Treatments	Earthworms (number m^{-2}) after harvest of second crop
T_1	11.58 (3.55)
T_2	11.95 (3.59)
T_3	10.99 (3.46)
T_4	12.25 (3.64)
T_5	10.94 (3.46)
T_6	11.95 (3.60)
T_7	12.62 (3.68)
T_8	13.31 (3.78)
SE	-
CD	-

Transformed values (($\sqrt{x+1}$ transformation) are given in parenthesis

CONCLUSION

The toxic effects of herbicides may affect the population of soil organisms at immediately after application. Gradually the population of these organisms increased and regained its original position within 30 days. A delay of 30 days in the restoration of normality after herbicide application should be considered normal with ecological consequences being negligible (Domsch*et al.,*1983). This steady level of microbial population and earthworm population recorded implies that the delicate biological balance of the soil is very little affected by application of PSE. On one hand, this result points to the very low residual effect of the herbicide and very low environmental hazard, and on the other, the reinstation of the beneficial microbial interaction that has a role to play in the protection and promotion of the crop.

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