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Dynamics of soil microbial population as influenced by post-emergence application of herbicide mixtures

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ABSTRACT

Soil microorganisms are the important link in soil-plant-herbicide-fauna- man relationship; their activity and diversity may serve as the bio indicators of soil health following herbicide application. Hence, bacterial, fungal and actinomycetes population were enumerated at just before herbicide application [15 days after sowing (DAS)], 30 days after herbicide application (45 DAS) and 45 days after herbicide application (60 DAS) during kharif 2014 and rabi 2014-15, with an objective to study the impact of post- emergence application of two pre-mix herbicide mixtures, bispyribac sodium + metamifop at four doses 60, 70, 80 and 90 g ha^{-1}, penoxsulam + cyhalofop butyl@120, 125, 130 and 135 g ha^{-1} on their population. The results revealed that compared to fungi and actinomycetes, bacterial population was found to be more at both the three stages of observation during both the years. Results also revealed that the tested dose of bispyribac sodium + metamifop (60, 70, 80 and 90 g ha^{-1}) and penoxsulam + cyhalofop butyl (120, 125, 130 and 135 g ha^{-1}) did not have any inhibitory effect on the population of bacteria, fungi and actinomycetes in direct seeded rice raised under puddled soil, implying the environmental safety of bispyribac sodium + metamifop and penoxsulam + cyhalofop butyl at their tested doses.

Keywords: Actinomycetes, bacteria, bispyribac sodium + metamifop, fungi, post-emergence herbicide, penoxsulam + cyhalofop butyl

Soil microorganisms play an important link in the soil-plant-herbicide-fauna-man relationship as they take part in the degradation of herbicides (Milosevic and Govedarica, 2002). Schloter *et al*. (2003) reported that soil microorganisms take part in various biochemical processes leading to the release of nutrients to the plants and are considered as the indicators of soil quality and health. These organisms have a vital role in maintaining the soil productivity; their number, activity and diversity may serve as the biological indicators of soil fertility (Rezende *et al*., 2004; Blagodatskaya and Kuzyakov, 2013). Devi *et al*. (2007) revealed that the reduction in the microbial population consequent to herbicide application is an indication of the harmful effect of herbicides to microflora. Change in soil microflora has been considered as one of the possible reasons for the decline in rice cropping systems (Reichardt *et al*., 1998). Herbicides can cause both qualitative and quantitative changes in the soil microbial population (Saeki and Toyota, 2004). Herbicides not only affect the target weed but also affect the soil microorganism by altering the metabolic activities (Singh and Walker, 2006) and physiological and biochemical behavior (Hussain *et al*., 2009). The increased dependence of herbicides for weed control in rice has led to concern about their toxicological behavior in rice field environment (Latha and Gopal, 2010). With this background, the present study was formulated to study the effect of microbial population consequent to the post-emergence application of two pre-mix herbicide combinations, bispyribac sodium + metamifop and penoxsulam + cyhalofop butyl for weed control in direct seeded puddled rice.

MATERIALS AND METHODS

Field experiments were conducted in farmers field during *kharif* 2014 and *rabi* 2014-15 in Kalliyoor Panchayat, Nemom Block, Thiruvananthapuram District, Kerala, India, situated at 8° 26.762' N latitude and 77° 0.136' E longitude and 29 m above mean sea level (MSL). Experiment was conducted in randomized block design with 12 treatments and three replications. The treatments comprised of bispyribac sodium + metamifop @ 60, 70, 80 and 90 g ha^{-1}(T_1, T_2, T_3, T_4) and penoxsulam + cyhalofop butyl @ 120, 125, 130 and 135 g ha^{-1}(T_5, T_6, T_7, T_8), bispyribac sodium alone 25 g ha^{-1} (T_9), penoxsulam alone 22.5 g ha^{-1}(T_{10}), hand weeding twice at 20 and 40 DAS (T_{11}) and weedy check (T_{12}). The herbicides were applied at 15 DAS as per the treatment schedule. The spray volume used in the study was 500 l ha^{-1} and herbicides were sprayed with hand operated knapsack sprayer fitted with a flat fan nozzle.

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In hand weeding treatment, weeding was done twice manually at 20 and 40 DAS. The soil of the experimental field was well drained sandy clay loam. Soil was acidic in reaction, high in organic carbon and medium in available N, P and K. The variety used for the study was Kanchana (PTB - 50), a short duration variety released from Regional Agricultural Research Station, Pattambi. The crop was uniformly fertilized with recommended dose of FYM (5 t ha^{-1}) and chemical fertilizers (70: 35: 35 kg N: P_2O_5: K_2O ha^{-1}). The entire dose of FYM was incorporated at the time of last ploughing. The fertilizers were applied in three splits; one third N and K and half P at 15 days after sowing (DAS), one third N and K and half P at 35 DAS and remaining one third N and K at 55 DAS.

The microbial enumeration studies were carried out at laboratory of Department of Agricultural Microbiology, College of Agriculture, Vellayani. Soil samples for the enumeration of total count of bacteria, fungi and actinomycetes were collected with soil auger just before herbicide application (15 DAS), 15 days after herbicide application (30 DAS) and 45 days after herbicide application (60 DAS). Four samples were collected from each treatment plot to a depth of 15 cm, mixed thoroughly to form a composite sample. The total count of bacteria, fungi and actinomycetes were assessed by serial dilution plate technique (Johnson and Curl, 1972). The media used for the estimation of bacteria, fungi and actinomycetes were nutrient agar, Martin's Rose Bengal agar and Kenknight's agar media, respectively and the dilution adopted were, 10^6, 10^4 and 10^5, respectively. Data were statistically analyzed and the treatment means were compared at 5 per cent probability level.

RESULTS AND DISCUSSION

Effect on bacterial population

Table 1: Effect of weed management treatments on population of soil bacteria

Weed management treatments	Population of soil bacteria (x 10^6 CFU g^{-1} wet soil)					
	Kharif 2014			*Rabi* 2014-15		
	JBHA (15 DAS)	15 DAHA (30 DAS)	45 DAHA (60 DAS)	JBHA (15 DAS)	15 DAHA (30 DAS)	45 DAHA (60 DAS)
Bispyribac sodium + metamifop @ 60 g ha^{-1}(T_1)	2.8	4.8	5.2	6.0	4.8	16.4
Bispyribac sodium + metamifop @ 70 g ha^{-1} (T_2)	2.5	4.6	4.9	6.9	4.8	14.4
Bispyribac sodium + metamifop @ 80 g ha^{-1} (T_3)	2.8	4.4	5.7	5.9	6.4	19.2
Bispyribac sodium + metamifop @ 90 g ha^{-1} (T_4)	3.1	5.3	6.9	6.2	7.6	19.6
Penoxsulam + cyhalofop butyl @ 120 g ha^{-1} (T_5)	3.0	4.2	7.2	5.9	7.2	14.4
Penoxsulam + cyhalofop butyl @ 125 g ha^{-1} (T_6)	2.9	4.9	5.5	6.0	9.9	16.0
Penoxsulam + cyhalofop butyl @ 130 g ha^{-1} (T_7)	2.9	4.5	6.1	6.2	9.2	20.0
Penoxsulam + cyhalofop butyl @ 135 g ha^{-1} (T_8)	3.1	8.5	5.7	6.1	11.3	18.4
Bispyribac sodium @ 25 g ha^{-1} (T_9)	3.1	4.4	4.1	6.2	5.6	13.7
Penoxsulam @ 22.5 g ha^{-1} (T_{10})	2.9	5.4	4.3	6.3	6.8	14.4
Hand weeding at 20 and 40 DAS (T_{11})	2.9	3.8	4.5	6.4	6.0	12.0
Weedy check (T_{12})	2.8	4.2	4.3	6.1	4.8	12.4
SEm (±)	**0.09**	**0.19**	**0.15**	**0.19**	**0.17**	**0.15**
LSD (0.05)	**NS**	**0.772**	**0.432**	**NS**	**0.489**	**0.447**

DAS - Days after sowing, JBHA - Just before herbicide application, DAHA - Days after herbicide application, NS - non significant

Results of the enumeration of bacterial population during both the years showed an increase in bacterial population at 15 days after herbicide application (30 DAS), except in bispyribac sodium + metamifop @ 60 and 70 g ha^{-1} during *rabi* 2014-15 (Table 1). Though reduction in bacterial population was observed in the above treatments, it was comparable with weedy check. Barman and Varshney (2008) opined that, generally in field condition, a very short initial depressive effect is noticed by herbicide application which was recouped fast. The increase in bacterial population might be because of the increase in the population of relatively resistant strains or due to the increase in the availability of nutrients either by the decomposition of the weeds or by the decomposition and degradation of applied herbicides. Sebiomo *et al*. (2011) also reported an increase in total bacterial population from 2nd week of herbicide application. After the 8th day of herbicide application, there a rapid increase in total bacterial population was observed in plots treated with pendimethalin, oxyfluorfen, and pretlilachlor (Trimurtulu *et al*., 2015).

Similar to observations at 30 DAS, at 60 DAS also an increase in population was observed in all the herbicide treatments except in penoxsulam + cyhalofop butyl @ 135 g ha^{-1}, bispyribac sodium applied alone and penoxsulam applied alone during first crop season. But these treatments also recorded bacterial population comparable or significantly higher than that of weedy check (control). These results imply that, the tested herbicides and their doses did not cause any adverse impact on soil bacteria, the most predominant group of microflorae in the soil. This is in conformity with the findings of Kalyanasundaram and Kavitha (2012) and Kumar *et al*. (2009) who observed that, the adverse effect of herbicides reduced gradually with passage of time and practically there was no impact on microbial population as a whole.

Effect on fungal population

Table 2: Effect of weed management treatments on population of soil fungi

Weed management treatments	Population of soil fungi (x 10^4 CFU g^{-1} wet soil)					
	Kharif 2014			*Rabi 2014-15*		
	JBHA (15 DAS)	15 DAHA (30 DAS)	45 DAHA (60 DAS)	JBHA (15 DAS)	15 DAHA (30 DAS)	45 DAHA (60 DAS)
Bispyribac sodium + metamifop @ 60 g ha^{-1}(T_1)	0.8	1.0	2.1	2.0	1.8	0.3
Bispyribac sodium + metamifop @ 70 g ha^{-1} (T_2)	1.1	1.2	2.4	2.2	2.6	0.2
Bispyribac sodium + metamifop @ 80 g ha^{-1} (T_3)	1.1	1.3	2.8	2.0	2.8	0.6
Bispyribac sodium + metamifop @ 90 g ha^{-1} (T_4)	1.0	1.7	3.3	2.3	1.5	0.8
Penoxsulam + cyhalofop butyl @ 120 g ha^{-1} (T_5)	1.0	2.3	1.8	2.3	1.4	0.2
Penoxsulam + cyhalofop butyl @ 125 g ha^{-1} (T_6)	1.1	2.7	1.7	2.6	3.0	0.8
Penoxsulam + cyhalofop butyl @ 130 g ha^{-1} (T_7)	1.2	2.7	1.5	2.3	3.1	0.1
Penoxsulam + cyhalofop butyl @ 135 g ha^{-1} (T_8)	1.3	2.0	3.3	2.3	3.2	1.2
Bispyribac sodium @ 25 g ha^{-1} (T_9)	1.1	1.3	2.7	2.1	2.1	0.1
Penoxsulam @ 22.5 g ha^{-1} (T_{10})	0.8	2.0	1.0	2.2	1.8	0.2
Hand weeding at 20 and 40 DAS (T_{11})	1.0	1.8	1.9	2.1	1.0	0.3
Weedy check (T_{12})	0.8	1.7	3.2	1.9	1.5	0.2
SEm (±)	**1.58**	**0.10**	**0.15**	**0.14**	**0.11**	**0.09**
LSD (0.05)	**NS**	**0.297**	**0.448**	**NS**	**0.327**	**0.265**

DAS - Days after sowing, JBHA - Just before herbicide application, DAHA - Days after herbicide application, NS - non significant

Fungal population was also significantly influenced by the herbicide treatments at 30 and 60 DAS during both the seasons (Table 2). During first crop season at 30 DAS, an increase in population was observed in all the treatments including weedy check and hand weeding as compared to observations at 15 DAS (just before herbicide application). During second crop season, a decrease in population was observed in weedy check, hand weeding, bispyribac sodium + metamifop @ 60 and 90 g ha^{-1}, penoxsulam + cyhalofop butyl @ 125 g ha^{-1} and penoxsulam applied alone @ 25 g ha^{-1}. Though a decline in fungal population was observed in these herbicide treatments, it was comparable with weedy check. The result is in line with the findings of Rajagopal (2013), who observed that at six days after the application of post emergence herbicide, azimsulfuron, the count of fungi showed substantial increase over weedy check and hand weeding treatment. Das *et al*. (2006) reported that fungi are known to be extremely adaptable in different environments due to their ability to break down many complex substances including herbicides. During first crop season, at 60 DAS, an increase in fungal population was observed in some treatments and a decrease in population in some other treatments. The herbicide treatments *viz*., penoxsulam + cyhalofop butyl@120, 125 and 130 g ha^{-1} which showed decline in population were also statistically comparable with hand weeding. Variation observed in the fungal population among the treatments might be due to the fact that, the herbicidal effects on fungal growth are specific to herbicide type and dose, microbial species and environmental condition (Bollen, 1961; Hattori, 1973). During second crop season, a decline in fungal population was observed in all treatments. The decline in fungal population might be due to the competition exerted by the tremendous increase in bacterial population (Table 1). These results indicated that, the applied herbicides and their tested doses had no adverse impact on soil fungi, the predominant microbial flora in the soil.

Effect on actinomycetes population

Table 3: Effect of weed management treatments on population of soil actinomycetes

Weed management treatments	Population of soil actinomycetes (x 10^5 CFU g^{-1} wet soil)					
	Kharif 2014			*Rabi* 2014-15		
	JBHA (15 DAS)	15 DAHA (30 DAS)	45 DAHA (60 DAS)	JBHA (15 DAS)	15 DAHA (30 DAS)	45 DAHA (60 DAS)
Bispyribac sodium + metamifop @ 60 g ha^{-1}(T_1)	2.5	1.5	2.3	4.2	2.2	2.1
Bispyribac sodium + metamifop @ 70 g ha^{-1} (T_2)	2.6	1.9	2.6	4..4	2.8	2.3
Bispyribac sodium + metamifop @ 80 g ha^{-1} (T_3)	2.8	1.8	2.5	4.3	2.6	2.0
Bispyribac sodium + metamifop @ 90 g ha^{-1} (T_4)	2.6	2.4	2.6	4.0	3.0	2.3
Penoxsulam + cyhalofop butyl @ 120 g ha^{-1} (T_5)	2.5	2.3	2.9	4.7	2.4	1.8
Penoxsulam + cyhalofop butyl @ 125 g ha^{-1} (T_6)	2.6	2.7	2.3	4.7	3.5	2.7
Penoxsulam + cyhalofop butyl @ 130 g ha^{-1} (T_7)	2.6	2.6	2.7	4.4	2.6	2.3
Penoxsulam + cyhalofop butyl @ 135 g ha^{-1} (T_8)	2.6	3.4	2.9	4.4	2.8	2.5
Bispyribac sodium @ 25 g ha^{-1} (T_9)	2.9	2.4	2.9	4.2	1.8	2.2
Penoxsulam @ 22.5 g ha^{-1} (T_{10})	2.8	3.4	2.5	4.4	2.2	2.0
Hand weeding at 20 and 40 DAS (T_{11})	3.0	2.9	3.0	4.9	2.8	2.5
Weedy check (T_{12})	2.8	2.2.	3.1	4.6	2.5	1.8
SEm (±)	**0.14**	**0.08**	**0.14**	**0.17**	**0.01**	**0.11**
LSD (0.05)	**NS**	**0.234**	**0.411**	**NS**	**0.293**	**3.08**

DAS - Days after sowing, JBHA - Just before herbicide application, DAHA - Days after herbicide application, NS - non significant

Contrary to fungal and bacterial population, reduction in population of actinomycetes was observed at 30 DAS (15 DAHA) in both the herbicide treated and non-treated plots (Table 3). Reduction in the population of actinomycetes might be due to competitive influence of various microorganisms on the population of actinomycetes in the rhizosphere as well as the toxic effect of the herbicides (Pal *et al*., 2013). Filimon *et al*. (2012) reported a decline in actinomycetes population after the application of sulfonyl urea herbicides. Observation at 60 DAS (45 DAHA) during first crop season indicated that, the population of actinomycetes was more as compared to 30 DAS. During second crop season, a reduction in population was observed in all the treatments. The reduction observed at 60 DAS during second crop season, might be due to the tremendous increase in bacterial population (Table 1). At 30 DAS during *kharif* 2014, bispyribac sodium + metamifop at 60, 70 and 80 g ha^{-1} registered significantly lower actinomycetes population compared to hand weeding, but during second crop season the difference was not statistically significant. At 60 DAS, during *rabi* 2014-15, bispyribac sodium + metamifop 60 and 70 g ha^{-1} and penoxsulam + cyhalofop butyl@125 g ha^{-1} showed significantly lower actinomycetes population as compared to non-herbicide treatment, hand weeding. However, the trend was not similar in both the seasons. Hence, the reduction in actinomycetes population observed in these treatments might be due to variation in edaphic factors, as reported by Singh and Singh (2009). Critical appraisal of the data on microbial population during both the seasons revealed no inhibitory action on microbial population by the application of herbicide mixtures *viz*., penoxsulam + cyhalofop butyl and bispyribac sodium + metamifop. Most microorganisms are capable of decomposing herbicides and using them most frequently as sources of biogenic elements for their own physiological process, which lead to an increase in microflora (Milosevic and Govedarica, 2002; Bera and Ghosh, 2013). Araujo *et al*. (2003) observed that an ideal herbicide should be degraded quickly into non-toxic substances and exerts lesser toxic effects on soil microbes.

Dynamics of soil microbial population consequent to the application of herbicide mixtures *viz*., bispyribac sodium + metamifop and penoxsulam + cyhalofop butyl revealed that the herbicide mixtures and their tested doses did not have any inhibitory effect on the growth of soil bacteria, fungi and actinomycetes at different stages of observation. Since, both the tested herbicide mixtures did not exert any harmful effect on soil microflora, it could be concluded that, both bispyribac sodium + metamifop and penoxsulam + cyhalofop butyl are ideal herbicides that degrade quickly to non-toxic substances and are environmentally safe.

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