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Impact of new herbicide molecule bispyribac sodium+metamifop on soil health under direct seeded rice lowland condition

SHEEJA K. RAJ*, ELIZABETH K. SYRIAC, L. GIRIJA DEVI, K. S. MEENAKUMARI[1], VIJAYARAGHAVAKUMAR[2] AND B. APARNA[3]

Department of Agronomy, College of Agriculture
Vellayani, Thiruvananthapuram-695 522 (Kerala), India
**(e-mail : sheejakraj70@gmail.com)*

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ABSTRACT

Soil microflora and soil enzymes are considered to be the biological indicators of soil health. Hence, an experiment was conducted in the farmer's field to study the impact of bispyribac sodium+metamifop 14% SE (a combination of broad spectrum herbicide bispyribac sodium 9.5% and a grass effective herbicide metamifop 3.8%) on soil microflora and enzyme activity in soil during **kharif** 2014. The experiment was conducted in randomized block design with seven treatments viz., bispyribac sodium+metamifop 14% SE applied at 60, 70, 80 and 90 g a. i./ha, bispyribac sodium 10% SC applied alone at 25 g a. i./ha, hand weeding twice on 20 and 40 days after sowing (DAS) and weedy check. The study results revealed that, on 15 days after herbicide application, an increase in the population of bacteria and fungi and decline in the population of actinomycetes was observed. But on 45 days after herbicide application, an increase in population of bacteria, fungi and actinomycetes was observed. In general, herbicides had no negative impact on total microbial population. An increase in dehydrogenase activity was observed in all herbicide treated plots on 15 days after herbicide application. The same trend was observed on 45 days after herbicide application. With regard to phosphatase activity irrespective of treatments decline in activity was observed on 15 days after herbicide application. On 15 and 45 days after herbicide application, bispyribac sodium applied at 70, 80 and 90 g a. i./ha was on par with weedy check. Similar to phosphatase, a decline in urease activity was observed on 15 days after herbicide application. At 15 and 45 days after herbicide application bispyribac sodium applied at 70, 80 and 90 g a. i./ha recorded higher urease activity than weedy check. From the results, it can be concluded that bispyribac sodium+ metamifop has no adverse impact on soil microflora and enzyme activity in the soil, implying that it is an ecofriendly herbicide.

Key words : Bispyribac sodium+metamifop, dehydrogenase, microbial population, phosphatase, soil pH, urease

INTRODUCTION

Soil micro-organisms and soil enzymes play a major role in soil fertility as they involve in the cycling of nutrients like carbon, nitrogen, phosphorus and sulphur which are required for the plant growth. They are the sensitive biological indicators of soil quality evaluation because they can sensitively reflect minute changes in the soil environment.

Soil microbial biomass is of great importance, because they play a crucial role in carbon flow, nutrient cycling and litter decomposition, which in turn affect soil fertility and plant growth (Bamboo *et al.*, 2013). Healthy population of bacteria, fungi and actinomycetes can stabilize the ecosystem. Any change in the population and activity may indirectly affect the nutrient cycling, which inturn affects the productivity, fertility and other soil functions (Wang *et al.*, 2008).

Soil enzymes, the vital activators in life processes, are known to play a substantial role in maintaining the soil health and its

[1]Department of Agricultural Microbiology.
[2]Department of Agricultural Statistics.
[3]Department of Agricultural Chemistry & Soil Science.

environment. They are important in catalyzing several vital reactions necessary for the life processes of micro-organisms in soils and the stabilization of soilstructure, the decomposition of organic wastes, organic matter formation and nutrient cycling (Dick, 1997). Soil enzymes often provide a unique integrative biological assessment of soil function, especially those that catalyze a wide range of soil biological processes, such as dehydrogenase, urease and phosphatase (Nannipieri *et al.*, 2002). The dehydrogenase enzyme activity is commonly used as an indicator of biological activity in soils (Burns, 1978). Phosphatase catalyzes hydrolytic break down of phosphomonoesters, thereby showing a high correlation between the content of soil phosphorus and an indicator of soil fertility. Urease is the enzyme that catalyzes the hydrolysis of urea to CO_2 and NH_4^+ ions by acting on C-N non-peptide bonds in linear amides. It is an important enzyme in soil that mediates the conversion of organic nitrogen to inorganic nitrogen and has been widely used to evaluate the changes in soil fertility (Nazreen *et al.*, 2012).

Herbicide usage seems to be inevitable in direct seeded rice, since weeds are the prime biological constraint due to the simultaneous emergence of rice and weed seedling, scarcity of labour and huge labour cost. A large number of pre- and post-emergence herbicides are used by the farmers to control weeds in rice. Latha and Gopal (2010) reported that the pre- and post-emergence application of herbicides resulted in large proportion of herbicides reaching the soil and accumulating in top 0 to 15 cm depth causing ecological damage. Preferred herbicides should not only have good efficacy, but also pose minimum adverse effects to crop, ecology and environment (Constenla *et al.,* 1990; Hoerlein, 1994).

The continuous use of herbicides with similar mode of action might lead to the development of resistance in certain weeds to the herbicides and cause shift in weed flora. One of the recent advent ways to overcome this problem is the use of herbicide mixtures. One such post-emergence herbicide mixture is bispyribac sodium+metamifop 14% SE. Metamifop is a grass effective herbicide of aryloxyphenoxy propionate group which inhibits the activity of acetyl coenzyme-A carboxylase and bispyribac sodium is from the group of pyrimidinyl carboxate which inhibits aceto lactatase synthase (ALS). Raj *et al.* (2013) reported that application of bispyribac sodium+metamifop 14% SE at 70 g a. i./ha was found more effective in controlling weeds than bispyribac sodium applied alone and also reported that application of double the dose (140 g a. i./ha) had no negative impact on crop growth. Though its bio-efficacy at different doses has been studied, its impact on soil enzymatic activity and microbial population has not been studied so far. Hence, a study was conducted with an objective to find out the impact of herbicide mixturebispyribac sodium+metamifop 14% SE on microbial population, dehydrogenase, urease and phosphatase activity in soil under field condition.

MATERIALS AND METHODS

A field experiment was conducted at farmers' field of Upanniyoor padashekharam, in Kalliyoor panchayat situated at a longitude and latitude of 8.5° N and 76.9° E and 29 m above MSL during **kharif** 2014 (May 2014 to September 2014). The experiment was conducted in randomized block design with seven treatments viz., bispyribac sodium+ metamifop 14% SE applied at 60, 70, 80 and 90 g a. i./ha, bispyribac sodium 10% SE applied alone at 25 g a. i./ha, hand weeding twice at 20 and 40 days after sowing (DAS) and weedy check. The soil was clay loam with pH 4.6, EC 0.2 dS/m, organic carbon 1.6%, available N 602.11 kg/ha, available P 24.64 kg/ha and available K 201 kg/ha.

The variety used for the study was "Kanchana" a short duration variety released from Regional Agricultural Research Station, Pattambi. The crop was sown on 30 May 2014. Seed rate adopted was 100 kg/ha. The crop was fertilized with 70 : 35 : 35 kg N : P_2O_5 : K_2O/ha with one-third N and K and half P applied on 15 DAS (after the soil sample collection for enzyme assay), one-third N and K and half P on 35th day and remaining one-third N and K on 55th day after sowing. Agronomic management practices were adopted as per Kerala Agricultural University Package of Practice recommendations (KAU, 2013) for raising the crop. The crop was harvested on 17 September 2014.

The average rainfall received during the cropping period was 892 mm with 46 numbers of rainy days and the mean maximum and minimum temperature recorded was 30.5° and 24.7°C, respectively.

The herbicides were applied on 15 DAS as per the treatment schedule. Herbicides were sprayed with the help of hand operated knapsack sprayer fitted with flat fan nozzle at a spray volume of 500 l/ha. In hand weeding treatment, two manual weedings were done on 20 and 40 DAS.

Soil samples for microbial enumeration and enzyme assay were collected with the help of soil auger just before herbicide application, 15 days after herbicide applicationand 45 days after herbicide application corresponding to seedling, maximum tillering and panicle initiation stage of the rice crop. Four samples were collected from each plot to a depth of 15 cm, mixed thoroughly to form a composite sampleand kept in a polythene bag and stored at 4°C. The microbial enumeration and enzyme assay were completed within a week. Bacterial, fungal and actinomycetes populationin the soil was enumerated by serial dilution technique (Timonin,1940) with nutrient agar, Martin's Rose Bengal and Kenknight's medium in 10^6, 10^3 and 10^4 dilutions. The dehydrogenase activity was assayed by quantifying the amount of triphenyl formazon produced and expressed as µg/g soil/24 h (Casida *et al.*,1964), urease activity in the soil was determined by quantifying the amount of urea hydrolyzedand expressed as µg/g soil/h (Watts and Crisp, 1954) andphosphatase activity was assayed by quantifying the amount p-nitrophenol released and expressed as µg/g soil/h (Eivazi and Tabatabai, 1977). The initial soil samples were analyzed by the following procedures. Soil reaction (pH) was determined in 1 : 5 soil water suspension (Jackson, 1973), electrical conductivity (EC) was determined in 1 : 5 soil water extract and expressed as dS/m (Jackson, 1973), organic carbon (OC) by wet digestion method (Walkley and Black, 1934) and expressed in %, available N by alkaline potassium permanganate (Subbiah and Asija, 1956) and expressed in kg/ha, available P by Bray No. 1 extract (Jackson, 1973) and expressed in kg/ha available K by neutral normal ammonium acetate (Jackson, 1973) and expressed in kg/ha. In addition to the initial soil pH, it was determined at three different time intervals from the soils collected for enzyme assay i. e. just before herbicide application, 15 days after herbicide application and 45 days after herbicide application. All data were statistically analyzed using ANOVA and difference between the treatment means was compared at 5% probability level.

RESULTS AND DISCUSSION

Effect on Soil pH

Data on soil pH revealed that just before herbicide application no significant variation was observed (Table 1) among herbicides. However, 15 days after herbicide application (corresponding to 30 days after sowing), decline in pH was observed in general in all the treatments. Significant variation in pH was observed among treatments with herbicide applied plots showing higher pH compared to non-herbicide plots (hand weeding twice and weedy check). The herbicide reaching the soil might be degraded by the micro-organism

Table 1. Soil pH as influenced by bispyribac sodium+metamifop 14% SE at different time interval

Treatment	Time interval		
	Juse before herbicide application	15 days after herbicide application	45 days after herbicide application
Bispyribac sodium+metamifop 14% SE @ 60 g a. i./ha	5.7	4.7	5.1
Bispyribac sodium+metamifop 14% SE @ 70 g a. i./ha	5.6	5.0	5.1
Bispyribac sodium+metamifop 14% SE @ 80 g a. i./ha	5.6	4.9	5.0
Bispyribac sodium+metamifop 14% SE @ 90 g a. i./ha	5.7	4.9	5.1
Bispyribac sodium 10% SE @ 25 g a. i./ha	5.6	5.0	5.3
Hand weeding twice at 20 and 40 DAS	5.4	4.8	5.0
Weedy check	5.4	4.6	5.2
Mean	5.6	4.8	5.1
C. D. (P=0.05)	NS	0.2	NS

NS : Not Significant.

producing organic metabolites which in turn might have enhanced the soil pH. Comparatively low pH observed at 15 days after herbicide application, irrespective of treatment, might be due the fact that only a thin film of water was maintained in the field from 20 to 30 DAS to enhance tillering. Draining and exposure to air reverse the pH changes in paddy soils (Dennett, 1932). After 30 DAS, the field was kept under submergence to a depth of 5 cm. Submergence will enhance the soil pH, this might be the reason for increased soil pH observed at 45 days after herbicide application (corresponding to 60 DAS). Ponnamperuma (1972) reported that submergence increased the pH of acid soil and depressed the pH of sodic and calcareous soils and converged the pH to 7.0.

Effect on Soil Microflora

The microbial load is the direct measurement of qualitative change after herbicide application. Data on microbial population indicated that just before herbicide application no significant variation was observed among different treatments. In general, bacteria were found in large numbers compared to fungi and actinonycetes (Table 2). The highest populations of bacteria, fungi and actinomycetes were observed in bispyribac sodium applied at 25 g a. i./ha. With the advancement of days after herbicide application, an increase in microbial population was observed. The result is in conformity with the finding of Singh and Singh (2009) who reported an increase in microbial population in groundnut crop 20 days after herbicide application. Data on the bacterial population at 15 days after herbicide application indicated an increase in bacterial population in herbicide applied plotscompared to weedy check. The increase might be due to the commensalic or protocooperative influence of various microorganisms on total bacteria in the rhizosphere soil (Bera and Ghosh, 2014). The same trend was noticed in fungal population also. The reason might be that micro-organisms take part in the degradation process of applied herbicides, and the degraded organic herbicides provide carbon rich substrates which in turn maximize the microbial population in the rhizosphere (Adhikary *et al.*, 2014). Though enhancement in fungal and bacterial population was observed on 15 days after herbicide application, actinomycetes population declined. This might be due to thecompetitive influence of various micro-organisms on the population of actinomycetes in the rhizosphere soil as well as toxic effect of the chemicals applied (Pal *et al.*, 2013) and also due to the decline in soil pH on 15 days after herbicide application (Table 1). Filimon *et al.* (2012) reported a decline in actiomycetes population after the application of sulfonylurea herbicidestribenuron-methyl and nicosulfuron. Increase in population of actinoycetes, bacteria and fungi observed on 45 days after herbicide application might be due to the increased availability of substrate for the micro-organism by the degradation of herbicide as well as nutrient supply. Careful appraisal of presented data revealed that total microbial population was not affected by the application of bispyribac sodium+metamifop at different doses ranging from 60 to 90 g a. i./ha. Bera and Ghosh (2013)

Table 2. Effect of treatment on viable count of microbial population (cfu/g of wet soil) as influenced by the herbicides

Treatment	Bacteria (cfu 10^6/g)			Actinomycetes (cfu 10^4/g)			Fungi (cfu 10^3/g)		
	JBHA	15 DAHA	45 DAHA	JBHA	15 DAHA	45 DAHA	JBHA	15 DAHA	45 DAHA
Bispyribac sodium+metamifop 14% SE @ 60 g a. i./ha	28	48	52	25	15	23	8	10	21
Bispyribac sodium+metamifop 14% SE @ 70 g a. i./ha	25	46	49	26	19	26	11	12	24
Bispyribac sodium+metamifop 14% SE @ 80 g a. i./ha	28	44	56	28	18	25	11	13	28
Bispyribac sodium+metamifop 14% SE @ 90 g a. i./ha	31	60	69	26	24	26	10	17	33
Bispyribac sodium 10% SE @ 25 g a. i./ha	31	43	51	30	24	29	11	13	27
Hand weeding twice at 20 and 40 DAS	29	38	45	29	29	30	10	18	19
Weedy check	28	42	43	28	22	31	8	17	32
Mean	29	46	52	27	22	27	10	13	24
C. D. (P=0.05)	NS	4.0	5.0	NS	3.0	5.0	NS	4.0	5.0

JBHA : Just before herbicide application, DAHA : Days after herbicide application. NS : Not Significant.

reported that micro-organisms were able to degrade herbicides and utilize them as a source of biogenic elements for their own physiological processes which leads to an increase of the soil microflora.

Dehydrogenase Activity

Soil dehydrogenase activity is considered as a valuable parameter for assessing the impact of herbicide treatments on the soil microbial biomass and can also be used as an indicator of the microbiological redox system.

There was no significant variation in dehydrogenase activity among treatments prior to herbicide application (Table 3). On 15 days after herbicide application results indicated a drop in dehydrogenase activity in hand weeding twiceand weedy check. The result is in agreement with the finding of Sebiomo *et al.* (2011) who observed that the application of herbicides to the soils led to a significant drop in dehydrogenase activity with respect to untreated control soil samples. It can also be observed that, compared to weedy check and hand weeding twice all the herbicide treatments recorded significantly higher dehydrogenase activity. This might be due to the greater availability of carbon source for the growth and activity of micro-organisms. The result is in close agreement with the findings of Rao *et al.* (2012). The highest activity observed in plot sprayed with bispyribac sodium+metamifop at 90 g a. i./ha might be due to the higher bacterial and fungal population. Brzeziñska (2006) observed a significant correlation between the dehydrogenase activity of soil and biomass of soil bacteria. Jezierska-Tys and Frac (2008) reported that dehydrogenase activity was also linked with the total fungal population. The lowest activity observed in hand weeding twice and weedy check might be due to the presence of lesser bacterial and fungal population (Table 2). The lower dehydrogenase activity observed in treatment bispyribac sodium+metamifop at 60 g a. i./ha and bispyribac sodium applied alone compared to other herbicide treatments might be due to less substrate availability. At 45 days after herbicide application, the treatment bispyribac sodium+metamifop applied at 60, 70, 80 and 90 g a. i./ha showed 7.42, 10.17, 10.91 and 12.93% increase in dehydrogenase activity, respectively, compared to weedy check. This indicated that the herbicide mixture bispyribac sodium+metamifop had no adverse impact on soil health and was found to be environmentally safe.

Phosphatase Enzyme Activity

Phosphatase is an exocellular enzyme produced by many soil micro-organisms that are responsible for the hydrolytic cleavage of a variety of ester-phosphate bonds of organic phosphates and anhydrides of orthophosphoric acid (H_3PO_4) into inorganic phosphate.

Careful appraisal of the presented data revealed reduction in phosphatase activity in all the treatment plots including the control plots on 15 days after herbicide application (Table 4). But on 45 days after herbicide application, increase in enzymatic activity was observed. This might be due to the change in species composition of soil micro-organisms as evident from Table 2 and variation in the availability of organic substrate. Kumar *et al.*

Table 3. Effect of herbicide bispyribac sodium+metamifop 14% SE on dehydrogenase activity (µg TPF released/g soil/day) in soil

Treatment	Juse before herbicide application	15 days after herbicide application	45 days after herbicide application
Bispyribac sodium+metamifop 14% SE @ 60 g a. i./ha	178.81	180.71	212.15
Bispyribac sodium+metamifop 14% SE @ 70 g a. i./ha	169.46	216.58	217.58
Bispyribac sodium+metamifop 14% SE @ 80 g a. i./ha	178.81	202.63	219.03
Bispyribac sodium+metamifop 14% SE @ 90 g a. i./ha	175.37	229.81	223.02
Bispyribac sodium 10% SE@ 25 g a. i./ha	178.63	186.06	189.41
Hand weeding twice at 20 and 40 DAS	184.17	102.08	183.53
Weedy check	169.57	113.60	197.49
Mean	176.40	175.92	206.03
C. D. (P=0.05)	NS	16.23	16.18

NS : Not Significant.

Table 4. Effect ofbispyribacsodoium+metamifopon acid phosphatase activity (µg of para nitro phenol released/g soil/h) in soil

Treatment	Just before herbicide application	15 days after herbicide application	45 days after herbicide application
Bispyribac sodium+metamifop 14% SE @ 60 g a. i./ha	48.60	28.62	29.76
Bispyribac sodium+metamifop 14% SE @ 70 g a. i./ha	50.73	34.07	34.44
Bispyribac sodium+metamifop 14% SE @ 80 g a. i./ha	51.08	30.62	41.24
Bispyribac sodium+metamifop 14% SE @ 90 g a. i./ha	50.85	34.82	38.73
Bispyribac sodium 10% SE @ 25 g a. i./ha	49.70	30.56	37.71
Hand weeding twice at 20 and 40 DAS	52.54	40.09	33.05
Weedy check	49.46	34.91	43.80
Mean	50.42	33.38	36.96
C. D. (P=0.05)	NS	2.53	7.01

NS : Not Significant.

(2007) reported that increase in phosphatase activity was mainly due to the presence of large fungal population and increased rate of hydrolysis of organic P in the soil.

Prior to herbicide application, no significant variation in phosphatase enzyme activity was observed among treatments with highest activity inhand weeding twice followed by bispyribac sodium+metamifop applied at 80 and 90 g a. i./ha. But on 15 days after herbicide application, significant variation in phosphatase activity was observed. The highest activity was observed in treatmenthand weeding twice followed by weedy check and bispyribac sodium+metamifop applied at 90 g a. i./ha. The reason might be due to the presence of more number of fungal colonies (Table 2). Nannipieri *et al.* (2011) reported that bacteria were the main source of alkaline phosphomonoesterase activity in soil, whereas acid phosphomonoesterase and phytase could derive from plants, fungi and bacteria. At 45 days after herbicide application, the highest activity was observed in weedy check which was on par with bispyribac sodium+metamifop applied at 80 and 90 g a. i./ha and bispyribac sodium applied alone at 25 g a. i./ha. The lowest phosphatase activity was observed in bispyribac sodium+metamifop applied at 60 g a. i./ha but it was found to be on par with hand weeding twice. The variation in phosphatase activity observed among the treatments might be due to the variation in the composition of the microflora and also the P availability and P demand by the crop. The results clearly demonstrated that the herbicide mixture bispyribac sodium+metamifop had no negative impact on the phosphatase activity in the soil, the enzyme which plays a major role in P transformation.

Urease Enzyme Activity

Urease enzyme catalyzes the hydrolysis of urea to ammonium and carbon dioxide. Ammonium formed represents a bioavailable form of nitrogen for plant uptake; this ubiquitous activity has a primary role in the cycling of nitrogen.

Similar tophosphatase enzyme activity,

Table 5. Urease activity in soil (µg of urea hydrolyzed/g soil/h) as influenced by bispyribac sodium+metamifop 14% SE

Treatment	Just before herbicide application	15 days after herbicide application	45 days after herbicide application
Bispyribac sodium+metamifop 14% SE @ 60 g a. i./ha	233.91	123.71	177.33
Bispyribac sodium+metamifop 14% SE @ 70 g a. i./ha	223.77	143.13	239.40
Bispyribac sodium+metamifop 14% SE @ 80 g a. i./ha	233.07	156.22	247.84
Bispyribac sodium+metamifop 14% SE @ 80 g a. i./ha	227.02	136.22	230.11
Bispyribac sodium 10% SE@ 25 g a. i./ha	233.06	116.11	250.80
Hand weeding twice at 20 and 40 DAS	235.73	129.62	178.16
Weedy check	233.20	134.27	182.82
Mean	231.39	134.18	215.21
C. D. (P=0.05)	NS	15.06	23.94

NS : Not Significant.

reduction in urease activity was observed on 15 days after herbicide application in all the plots including weedy check and hand weeding twice. The decline in urease activity observed on 15 days after herbicide applicationmight be due to the variation in soil properties like soil pH (Table 1) and also due to the temporary inhibition caused by the metabolites produced as a result of degradation of herbicides by the micro-organisms. The increase in urease activity observed on 45 days after herbicide application was owing to the fact that time advanced, temporary inhibition in enzyme activity was overcome by the microflora with the increase in microbial population (Table 2).More the microbial biomass, more the exudation resulted in higher enzymatic activity (Lu-Sheng *et al.*, 2005) and also due to the increasein soil pH (Table 1). The bispyribac sodium+metamifop applied @ 70, 80 and 90 g a. i./ha recorded higher urease activity than that of weedy check and hand weeding twice, might be due to the increased substrate availability. Bispyribac sodium+metamifop applied at 60 g a. i./ha recorded lower urease activity than its higher doses but it was on par with hand weeding twice (Table 5). This clearly indicated that bispyribac sodium+metamifop applied at 60 to 90 g a. i./ha had no adverse effect on urease activity in the soil.

CONCLUSION

From the study it can be concluded that the application of new herbicide molecule bispyribac sodium+metamifop at 60 to 90 g a. i./ha has no adverse effect on the microbial population as well as enzyme activity (dehydrogenase, phosphatase and urease activity), the biological indicators of soil health. Since it has no negative impact on microflora and soil enzyme activity, bispyribac sodium+metamifop can be considered as an environmentally safe herbicide.

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