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Microbial and dehydrogenase activity of soil contaminated with herbicide combination in direct seeded rice (*Oryza sativa* L.)

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Abstract
An attempt was made in the present investigation to study the effect of new herbicide combination molecules like bispyribac sodium, metamifop, almix, clincher on rhizosphere soil microflora of direct seeded rice (DSR) at the research farm of Wetland Farm, Tamil Nadu Agricultural University, Coimbatore, India. The present study results showed that the herbicide treatments significantly inhibited the development of microbial populations in the soil and the degree of inhibition varied with the types of herbicide. Increasing trend of inhibition on growth of microbial populations was observed from the initial effect until 30 days after herbicide spray (DAHS). No inhibition was observed at 30 DAHS to harvest stage. A gradual raise was observed in the population of micro-organism like bacteria, fungi and actinomycetes with the application of herbicide combination in the rhizosphere soil of DSR. Among different herbicide treated plots, post emergence (POE) application of bispyribac sodium 4% SE + metamifop 10% SE at 70, 56 and 42 g a.i./ha + wetter at 100 ml/ha recorded maximum microbial populations and dehydrogenase activities. The study suggests that the herbicide application to soil cause transient impacts on microbial population growth, when applied at recommended field application rate.

Keywords: DSR, weed density, grain yield, microorganism, dehydrogenase activity

1. Introduction
Rice (*Oryza sativa* L.) is the leading cereal of the world and more than half of the human race for their daily sustenance [1]. Globally, actual rice yield losses due to pests have been estimated at 40%, of which weeds has the highest loss potential of 32%. The worldwide estimated loss in rice yield from weeds is around 10% of the total production [2]. To meet the global rice demand, it is estimated that about 114 million tonnes of additional milled rice need to be produced by 2035 which is equivalent to an overall increase of 26% in the next 25 years [3]. To sustain present food self-sufficiency and to meet future food requirements, India has to increase its rice productivity by 3% per annum but the possibility of expanding the area under rice in the near future is limited. There has, however, been stagnation in rice productivity in recent years and long-term experiments showed a declining trend in rice yield. Due to receding water table, rising costs of labour for transplanting of paddy and the adverse effects of puddling on soil health; direct seeded rice (DSR) is gaining popularity. But, weeds are the main constraint for farmers practising direct seeding so use of herbicides both pre- and post-emergence is required for good crop. An unintended consequence of the application of herbicides is that it may lead to significant changes in the populations of microorganisms and their activities thereby influencing the microbial ecological balance in the soil [4, 5] and affecting the productivity of soils. When herbicides are applied in soil, they may exert certain side effects on non-target organisms. Therefore, there has been considerable interest on the influence of herbicides on the soil microflora and microbially mediated processes. The effects of these chemicals on certain variables are associated with microbial biomass and their activity. The increasing reliance of rice cultivation on herbicides has led to concern about their eco-toxicological behaviour in the rice field environment. Soil health and microbial diversity have become vital issues for the sustainable agriculture. Loss of microbial biodiversity can affect the functional stability of the soil microbial community and soil health. Generally, there are some negative effects of herbicides on the population level or composition of species. The impact of applied herbicides on the soil microbial populations were studied which included analysis of bacteria, fungi and actinomycetes counts. Dehydrogenase is thought to be an indicator of overall microbial activity because it occurs intercellularlly in all living microbial

cells and is linked with microbial oxydoreduction processes [6, 7]. It is a specific kind of enzyme which plays significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors. Soil dehydrogenase activity is considered to be a value parameter for assessing the side effects of herbicides treatments on the soil microbial biomass. In Tamil Nadu, several post-emergence herbicides are being used in DSR for chemical weed control; therefore, this work was carried out to estimate the counts of these microbes at different period of crop growth after their application.

2. Materials and Methods

2.1 Experimental Site and Initial Soil Characteristics

A field study was conducted for two years (*Rabi* seasons of 2013 and 2014) at the research farm of Wetland Farm, Tamil Nadu Agricultural University, Coimbatore, India. The experimental farm was located in Western Zone of Tamil Nadu is at 11º29"N latitude and 77º08"E longitude with an altitude of 256 m above MSL. The climate is semi arid, with an average of 674.2 mm distributed over 47 rainy days (mean of past 50 years). The maximum rainfall received during the cropping period was 70 mm. During the cropping period, the maximum and minimum temperature ranged from 35.7 to 27.0ºC and 26.0 to 19.8ºC, respectively. Relative humidity ranged from 61 to 95% and 29 to 75% during forenoon and afternoon, respectively. The solar radiation received during the cropping period ranged from 224 to 462.6 cal/cm^2/day and the sunshine hours ranged from 1.4 to 9.0 hrs/day. The evaporation prevailing during the cropping period ranged from 2.4 to 9.2 mm. The soil was clay loam in texture with low in available nitrogen (238 kg/ha), medium in available phosphorus (16.8 kg/ha) and high in available potassium (518 kg/ha) with 0.5% organic matter with a pH of 7.4.

2.2 Experimental design and treatments

The treatments in each year were arranged in a randomized complete block design with three replications. Twelve weed control treatments were included with different doses of herbicide combination options for weed control in DSR. Herbicides included in the study were bispyribac sodium (nominee gold), metamifop, almix (chlorimuron + metsufuron 20% WP), clincher (cyhalofop buthyl 10% EC) and wetter (isoxadifen).

2.3 Experimental details, selection of cultivar and sowing

In each year, rice (cv. ADT 43, a cultivar with the duration of 120 days) was seeded in the first week of September and the harvested in last week of December. Manually operated rice drum seeder developed by Tamil Nadu Agricultural University, Coimbatore was used for sowing the seeds. The seeder has two wheels at both the ends. It drops the seeds at 30 cm apart in continuous row. At a time, eight rows of rice seeds were sown. A seed rate of 40 kg/ha was adopted. Before sowing the field was drained to keep it under saturated condition to facilitate easy sowing and uniform establishment of seedlings. A thin film of water was maintained at the time of sowing. For the next 8-15 days, irrigation and drainage of water were alternated to facilitate aeration, adequate moisture for germination of seed and establishments of seedlings. Thereafter, the plots were irrigated to 2 cm depth uniformly in all the treatments after the appearance of hair line cracks, upto panicle initiation stage. After panicle initiation, the plots were irrigated to 5 cm depth on disappearance of ponded water. Irrigation was stopped 15 days prior to harvest.

2.4 Treatment details

New formlation of herbicide combination bispyribac sodium 4% SE + metamifop 10% SE was applied as POE herbicide on 10 to 15 DAS (Table 1). Bispyribac sodium has been widely used for DSR with its excellent foliar efficacy against grasses, sedges and broad leaved weeds. Metamifop which was discovered by Dongbu Honnong Co., Ltd. is a novel grass herbicide with excellent foliar efficacy against grasses and crop safety. Hand operated knapsack sprayer fitted with a flat fan type nozzle (WFN 40) was used for spraying the herbicides adopting a spray volume of 500 litres ha^{-1} in DSR. The herbicides were sprayed by keeping a thin film of water in the field. The field was neither drained nor irrigated for 2 days after application of herbicides. The non-treated control plot was kept undisturbed for the entire cropping period.

Table 1: Herbicide treatments used in the study

Tr. No	Treatment details	Dose g.a.i/ha	Dose ml/gm/ha of Formulation	Time of Application
T_1	Bispyribac sodium 4% SE + metamifop 10% SE + wetter	42 g a.i. + 100 ml wetter	300 ml + 100 ml wetter	10-15 DAS
T_2	Bispyribac sodium 4% SE + Metamifop 10% SE + wetter	56 g a.i. + 100 ml wetter	400 ml + 100 ml wetter	10-15 DAS
T_3	Bispyribac sodium 4% SE + Metamifop 10% SE + wetter	70 g a.i. + 100 ml wetter	500 ml + 100 ml wetter	10-15 DAS
T_4	Bispyribac sodium 4% SE + Metamifop 10% SE + wetter	140 g a.i. + 100 ml wetter	1000 ml + 100 ml wetter	
T_5	Almix (Chlorimuron + Metsufuron 20% WP)	4 g a.i.	20 g	10-15 DAS
T_6	Clincher (Cyhalofop Buthyl 10% EC)	80 g a.i.	800 ml	10-15 DAS
T_7	Bispyribac sodium 10% SC + wetter	20 g a.i. + 100 ml wetter	200 ml + 100 ml wetter	10-15 DAS
T_8	Metamifop 10% SE + wetter	50 g a.i. + 100 ml wetter	500 ml + 100 ml wetter	10-15 DAS
T_9	Bispyribac sodium 4% SE + Metamifop 10% SE	70 g a.i.	500 ml	10-15 DAS
T_{10}	Bispyribac sodium 10% SC	20 g a.i.	200 ml	10-15 DAS
T_{11}	Metamifop 10% SE	50 g a.i.	500 ml	10-15 DAS
T_{12}	Hand weeding twice on 25 and 45 DAS	---	---	---
T_{13}	Non-treated control	---	---	---

Abbreviation: DAS - Days after sowing

2.5 Observation on weeds
2.5.1 Weed flora of the experimental field
To account for the general weed flora of the experimental field, species wise weeds observed in the treatment plots were recorded during the period of maximum appearance of 20 and 40 DAHS. The weed flora of the experimental site was recorded species wise.

2.5.2 Weed density
The weed count was recorded species wise using 0.5 m x 0.5 m quadrant from four randomly fixed places in each plot and the weeds falling within the frames of the quadrant were counted and the mean values were expressed in number/m^2. The density of grasses, sedges and broad leaved weeds and also the total weeds were recorded at 20 and 40 DAHS and expressed in number/m^2.

2.5.3 Weed control efficiency
Weed control efficiency (WCE) was calculated as per the procedure [8].

$$WCE\ \% = \frac{WD_c - WD_t}{WD_c} \times 100$$

Where,
WCE - weed control efficiency (%)
WD_c - weed biomass (g/m^2) in control plot
WD_t - weed biomass (g/m^2) in treated plot

2.6 Observation on crop
2.6.1 Grain yield
Grains from each net plot were cleaned; sun dried, weighed and adjusted to 14% moisture content and the grain yield was expressed in kg/ha.

2.7 Microbial analysis
2.7.1 Enumeration of microbial population
Soil samples from different treatments were analyzed for enumeration of total bacteria, fungi and actinomycetes. Enumeration was done by employing serial dilution and plating technique with appropriate media. Tenfold serial dilution was prepared for each soil sample till appropriate dilution was obtained.

2.7.1.1 Bacterial population
Enumeration of bacteria was carried out in soil extract agar medium using standard dilution plating technique. One g of soil sample was serially diluted by 10 fold series using sterile water blank up to 10^{-8} and one ml of aliquot from 10^{-8} dilution was taken and dispensed in sterile petriplates with soil extract agar medium. The bacterial colonies were enumerated after 48 hrs of incubation at 37°C and expressed as number of cfu/g dry weight of soil [9].

2.7.1.2 Fungal population
Enumeration of fungi was carried out in Rose Bengal Agar medium. One g of soil sample was serially diluted by 10 fold series using sterile water blank up to10^{-5} and one ml of aliquot from 10^{-5} dilution was taken and dispensed in sterile petriplates with Rose Bengal Agar medium. The fungal colonies were enumerated after 5 days of incubation at 37°C and expressed as number of cfu/g dry weight of soil [10].

2.7.1.3 Actinomycetes population
Enumeration of actinomycetes was carried out in Kenknight's Agar medium. One g of soil sample was serially diluted by 10 fold series using sterile water blank up to10^{-5} and one ml of aliquot from 10^{-5} dilution was taken and dispensed in sterile petriplates with Kenknight's Agar medium. The actinomycetes colonies were enumerated after 7 days of incubation at 37°C and expressed as number of cfu/g dry weight of soil [11].

2.8 Soil enzyme activity
2.8.1 Dehydrogenase
Twenty gram of dry soil (particle size < 0.02 mm) was mixed with 0.2 g of $CaCO_3$ splitted in to three boiling tube. To this, 1ml of 3% 2, 3, 5 - triphenyl tetrazolium chloride and 2.5 ml distilled water was added, mixed thoroughly, stoppered the tube and incubated for 24 h at 37°C. After incubation, 10 ml methanol was added and stoppered the tube and mixed for 1 min, unstoppered the tube, sample was then filtered using a glass funnel plugged with absorbent cotton. The tube was washed with methanol and soil was transferred in to glass funnel. After this, methanol was added in to funnel until the reddish colour disappeared from the cotton plug. Filtrate was diluted to 100 mL with methanol. The filtrate obtained was red in colour. The intensity of red colour was measured immediately in spectrophotometer at 485nm. The concentration of dehydrogenase in the sample was obtained from the standard graph drawn by using Tri Phenyl Formazan (TPF) as standard, and expressed as µg of TPF released per g soil on dry weight basis [12].

2.9 Statistical analysis
The data on various characters studied during the course of investigation were statistically analyzed as suggested by [13]. Data on weed density and dry weight showed high variation and were subjected to square root transformation of $\sqrt{(X+2)}$ and analyzed statistically. Wherever statistical significance was observed, critical difference (CD) at five per cent level of probability was worked out for comparison. Non- significant comparison was indicated as 'NS'.
Treatments where the differences are not significant were denoted as NS.

3. Results and Discussion
3.1 General weed flora of the experimental field
A critical analysis of relative proportion of grasses, sedges and broad leaved weeds to total weed population in non-treated control revealed that during the crop growth period, the population of sedges was higher than that of grasses and broad leaved weeds. Among the grasses, *Echinochloa crus-galli* (L.) Beauv., *Echinochloa colona* (L.) Link., *Dinebra retroflexa* (Vahl.) Panzer. and *Panicum repens* (L.) were the dominant species and major sedges were *Cyperus difformis* (L.), *Cyperus irria* (L.) and *Fimbristylis miliacea* (L.) Vahl. Among the broad leaved weeds M*arsilea quadrifoliata* (Linn.), *Ammania baccifera* (L.) and *Eclipta alba* (L.) Hassk. were the dominant species. However, a species-wise result was given for the first five weeds only, as they were the predominant weeds in the experimental trial.

3.2 Total weed density
During both the years, lesser total weed density was observed with POE application of bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter at 100 ml/ha and bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha without wetter and it was closely followed by application of bispyribac sodium 4% SE + metamifop 10% SE at 56 g a.i./ha + wetter at 100 ml/ha (16.80, 17.09, 22.50 in 2013 and 13.90,

15.43, 18.44 in 2014, respectively). At 40 DAHS also similar results were recorded (Table 2). Bispyribac sodium is pyrimidinyl carboxate group which inhibits the biosynthesis of amino acids. Metamifop is aryloxyphenoxy propionate group which inhibits the activity of acetyl coenzyme-A carboxylase (ACCase) leading to growth retardation of weeds. However, the combined application of both herbicides induces chlorosis selectively in weeds and insufficient chlorophyll production makes it difficult for thrive of weeds. The combined application of these herbicides was better than their individual application in reducing the weed density, weed biomass and enhancing the productivity of rice yield. Total weed density was higher in individual application as POE application of clincher at 80 g a.i./ha when compared to almix at 4 g a.i./ha and it was similar in the both years of study. Clincher is a systemic POE herbicide and it is aryloxyphenoxy propionate group. In the present study, POE applications of clincher (alone) effectively control grassy weeds than compared to sedges and broad leaved weeds in direct seeded rice. Total weed density in the non-treated control were 105.20 and 156.13 plants/m^2 in 2013; 85.93 and 1132.78 plants/m^2 in 2014, respectively at 20 and 40 DAHS. All the herbicide treatments recorded lower total weed density significantly as compared to the non-treated control. Sequential applications of pre and post-emergence herbicides provided better weed control than the sole application pre or post-emergence herbicides in DSR [14].

3.3 Weed control efficiency

Adoption of herbicide combination of bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter at 100 ml/ha treatment exhibited lower weed infestation with higher weed control efficiency than sole herbicide application in the present study. During both the years, it was observed that POE application of herbicide combination bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter at 100 ml/ha resulted the higher weed control efficiency of 87.43 and 80.07% in 2013 and 88.45 and 81.68%, in 2014, respectively and it was followed by application of bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i. ha^{-1} without wetter (86.55 and 73.10% in 2013 and 86.35 and 80.08%, in 2014, respectively). In the present study at 40 DAHS, weed control efficiency with ranged from 47.89 to 66.06% (single herbicide application); 60.22 to 80.07% (new herbicide combination) in 2013, respectively and weed control efficiency ranged from 55.67 to 66.48% (single herbicide application); 63.14 to 81.68% (new herbicide combination) in 2014, respectively (Table 2).

3.4 Response of grain yield

Rice grain yield following all herbicide treatments ranged from 4276 to 5676 kg/ha and 4658 to 6388 kg/ha, while the non-treated control plots yield of 2734 and 3012 kg/ha in 2013 and 2014, respectively (Table 2). Higher grain yield was recorded in the plots treated with the POE application of new formulation bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter at 100 ml/ha (5676 kg/ha in 2013 and 6388 kg/ha in 2014) and it was similar to the grain yield observed in the plots treated with the application of herbicide combination of bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha without wetter (5488 kg/ha in 2013 and 6232 kg/ ha in 2014), bispyribac sodium 10% SC at 20 g a.i./ha + wetter at 100 ml/ha (5442 kg/ha in 2013 and 6076 kg/ha in 2014) and hand weeding twice on 25 and 45 DAS (5256 kg/ha in 2013 and 5908 kg/ha in 2014). Bispyribac sodium 4% SE + metamifop 10% SE showed on par with hand weeding twice on 25 and 45 DAS for most of the yield parameters and grain yield. These treatments recorded lesser crop weed competition during the critical period of rice that was marked as more panicles per unit area, increased grain number and grain weight over non-treated control. Higher grain yield in response to efficient weed control [15, 16, 17]. The present study data showed effectiveness of manual weeding in limiting weed density and dry biomass merely owing to POE application of new herbicide combination as an effective tool for their weed management in direct seeded rice. Nonetheless, during later part of the growing season weeds were also suppressed by shading effect of rice in manually weeded plots due to quick and dense canopy closure [18]. In both the years, grain yield in the plots treated with already existing molecule of almix at 4 g a.i./ha (4948 kg/ha in 2013 and 5792 kg/ha in 2014) and clincher at 80 g a.i. ha^{-1} (4404 kg/ha in 2013 and 5248 kg/ha in 2014) was similar, but lower than grain yield recorded in the bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter. Though, the combined application of bispyribac sodium 4% SE + metamifop 10% SE + wetter with all different doses were very effective, provide broad spectrum weed control and subsequently increasing the productivity of direct seeded rice in this study. POE application of bispyribac sodium + metamifop 14% SE at 70 g/ha with wetter can keep the total weed density and weed biomass reasonably at lower level and enhance the productivity of DSR [19].

3.5 Effect of herbicide residue on soil micro-organisms

In the present study, the microbial population was inhibited immediately after the application of herbicides due to toxicity in soil environment. Microbial count recorded at 30 DAHS showed that the herbicide did not influence significantly the number of bacteria, fungi and actinomycetes. But, at later stage of 30 days after application of herbicide lost their potency, probably due to their degradation in soil. As herbicides occupy 47% of the agrochemicals usage, there could be a chance for the bioaccumulation and biomagnification of the compounds or its metabolites in the crop, which may cause ailing effects to human being through food chain [20].

3.5.1 Herbicides combination on soil bacteria

Bacterial population was significantly influenced by weed control practices during the initial stages immediately after herbicide spray while, after 30 DAHS no marked variation among the treatments was noticed. Among the herbicidal treatment plots, POE application of bispyribac sodium 4% SE + metamifop 10% SE at 140 g a.i./ha + wetter at 100 ml/ha recorded lower bacterial population upto 15 DAHS and it was comparable with metamifop 10% SE at 50 g a.i./ha. In the remaining stage of observation the POE herbicides lost their effect due to their degradation in soil. (Table 3). For all the cases of herbicidal treatments, total bacteria recovered from initial loss and exceeded the initial counts [21]. Microbial populations in the herbicide applied plots were more or less similar to the hand weeding twice and unsprayed control and thus indicating that herbicides have no detrimental effect on soil health at the applied doses. Microbial activities were more sensitive to pesticides than population densities. Also, pesticide degradation in rice fields was favoured by high temperatures which usually stabilize in range favouring high microbial activity and further accelerated by organic matter incorporation. In the intial stage, decrease in the population of total bacteria was due to competitive influence and the toxic effect as well as different persistence periods of the treated herbicides in different soil environments. In addition, the increase population was affected by the commensalic or proto-cooperative influence of various micro-organisms on total bacteria in the rhizosphere soil of direct seeded rice.

3.5.2 Herbicides combination on soil fungi

Weed management methods exerted significant influence on the fungi population during all the stages of observation except at 15 DAHS and harvest stage. POE application of bispyribac sodium 4% SE + metamifop 10% SE at 140 g a.i./ha + wetter at 100 ml/ha registered significantly lower fungi population over rest of the treatments at all the stages. It was comparable with application of POE application of clincher (cyhalofop buthyl 10% EC) at 80 g a.i./ha. Unsprayed control and herbicide combination applied plots with wetter registered higher population of fungi which were significantly superior over the rest of the individual herbicide applied plots (Table 3). The inhibition of fungi growth is dependent on chemical nature of the herbicides. At recommended field application rate, these herbicides could be considered as only moderately toxic to the fungal colony development, causing moderate inhibition of 40%. This indicated that applications of the herbicides at the optimum rate could be moderately detrimental to the fungal development in soil. Fungal colonies therefore showed their ability to recover from the toxic effect by 15 DAHS and at 30 DAHS, no further inhibition or full colony recovery was observed at later stage. Micro-organisms are able to degrade herbicides and utilize them as a source of biogenic elements for their own physiological processes [22]. However, before degradation, herbicides have toxic effects on micro-organisms, reducing their abundance, activity and consequently, the diversity of their communities.

3.5.3 Herbicides combination on soil actinomycetes

The effect of weed control methods on the population of actinomycetes followed a similar trend as that of bacterial population and significant variation was observed during early stages, while, after 15 DAHS, weed management practices failed to exert marked variation on the population of actinomycetes. POE application of bispyribac sodium 4% SE + metamifop 10% SE at 140 g a.i./ha + wetter at 100 ml/ha very much restricted the growth of actinomycetes and recorded lower population in the initial stages. Actinomycetes population started to recover slowly after 15 DAHS irrespective of the treatments. Like the bacteria and fungi, variation in actinomycetes population was recorded between herbicides treated plots and the hand weeding or the control plots upto 15 DAHS (Table 4). Thereafter the population increased significantly than the unsprayed control. Significantly higher microbial populations in the herbicidal treatments at all stages of observation might be due to healthy and conducive environment for the micro-organisms as compared to the control. No particular pattern of the microbial counts was observed among weed control treatments but the microbial counts were significantly lower in unsprayed control. It may be showed that there was increase in the biological properties of the soil in well aerated aerobic soil conditions found in DSR hence might be ascribed to the improvement in the nutrient status as well as physical conditions of the soil which resulted in better growth of the micro-organisms. It could be further inferred that the microbial population started to regain after the weeds were also killed by the herbicides and got mixed in the soil during this period and these might have served to increase the nutrients. The degradation of herbicides may be serving as carbon source for growth of microbes.

3.6 Herbicides combination on dehydrogenase activity

Dehydrogenase activity study revealed that in comparison to different herbicides treated plots, there was a continuous increment of dehydrogenase activity in unsprayed control from 0 to 60 days after transplanting followed by a slight decrement upto harvest. It was observed that all the herbicides including wetter significantly inhibited the dehydrogenase activity after their application at 15 DAHS in DSR. Herbicide treatment resulted in a significant drop in dehydrogenas activity when compared to the unsprayed control of soil samples. Attained results indicated that soils treated with bispyribac sodium 4% SE + metamifop 10% SE at 140 g a.i./ha + wetter at 100 ml/ha recorded lower dehydrogenas activity of 56.79 µg TPF released/g dry weight of soil when compared to other herbicidal treatments. Unsprayed control and hand weeding twice registered maximum dehydrogenas activity (122.70 and 114.68 µg TPF released /g dry weight of soil) and it was on par with bispyribac sodium 4% SE + metamifop 10% SE at 42, 56 g a.i./ha + wetter at 100 ml/ha and closely followed by bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter. Among different herbicide treated plots, bispyribac sodium 4% SE + metamifop 10% SE at 42 g a.i./ha + wetter at 100 ml/ha recorded maximum dehydrogenase activities of 81.56 µg TPF released /g dry weight of soil (Table 4). This might be due to lesser toxicity of herbicide molecules over microbes. Dehydrogenase is thought to be an indicator of overall microbial activity, because it occurs intercellular in all living microbial cells and is linked with microbial oxydo reduction processes [6, 7]. Dehydrogenase activity was lower with the increasing dose of bispyribac sodium 4% SE + metamifop 10% SE. Among the different herbicidal treatments, clincher (cyhalofop buthyl 10% EC) at 80 g a.i./ha exhibited maximum inhibition in dehydrogenase activities when compared to metamifop 10% EC. Generally, dehydrogenase activity increased from the second to the sixth week of the herbicidal treatment. This might due to increase in microbial populations with the capability of utilizing the herbicides as carbon source [23]. Dehydrogenase activity is a sensitive bioindicator of the microbial activity response to herbicide inputs. Above observations were in close agreement with Nowark (1996) and Wyszkowska and Kucharski (2004) who claimed that dehydrogenase are an objective reflection of the biological state of soil [24, 25]. They also reported that higher rate of triflurotox depressed the dehydrogenase activity by 28.7% before sowing and 35.6 to 72.6% after harvest of crop, in comparison to the control. The inhibitory effect of herbicides on the activity of dehydroenase has also reported by Strzelec (1986) and Pietr and Jablonska (1987) [26, 27].

Table 2: Total weed dry weight, weed control efficiency and grain yield as influenced by different weed management practices in direct seeded rice

Treatments	Total weed density (No./m²), WCE (%) & grain yield (kg/ha)									
	rabi, 2013					*rabi*, 2014				
	Total weed density		WCE		Grain yield	Total weed density		WCE		Grain yield
	20 DAHS	40 DAHS	20 DAHS	40 DAHS		20 DAHS	40 DAHS	20 DAHS	40 DAHS	
T_1 - Bispyribac sodium 4% SE + metamifop 10% SE at 42 g a.i./ha + wetter	5.83 (32.03)	6.87 (45.17)	67.33	60.22	4286	5.07 (23.68)	7.86 (59.80)	72.49	63.14	4658
T_2 - Bispyribac sodium 4% SE + metamifop 10% SE at 56 g a.i./ha + wetter	4.95 (22.50)	5.98 (33.81)	76.37	69.73	4978	4.52 (18.44)	6.73 (43.35)	81.17	72.11	5722
T_3 - Bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter	4.43 (16.80)	5.27 (25.78)	87.43	80.07	5676	3.99 (13.90)	5.12 (24.19)	88.45	81.68	6388
T_4 - Almix (Chlorimuron + Metsufuron 20% WP) at 4 g a.i./ha	6.75 (43.61)	7.79 (58.76)	65.60	62.63	4948	6.01 (34.13)	8.07 (63.14)	71.14	65.08	5792
T_5 - Clincher (Cyhalofop Buthyl 10% EC) at 80 g a.i./ha	7.51 (54.47)	6.99 (46.88)	62.25	58.36	4404	6.04 (34.54)	8.43 (69.06)	69.12	66.48	5248
T_6 - Bispyribac sodium 10% SC at 20 g a.i./ha + wetter	5.84 (32.12)	7.50 (54.26)	69.62	66.06	5442	5.81 (31.70)	6.86 (45.05)	75.01	65.64	6076
T_7 - Metamifop 10% SE at 50 g a.i./ha + wetter	6.81 (44.41)	6.79 (44.08)	63.32	50.22	5004	6.09 (35.13)	7.44 (53.29)	71.40	61.51	5748
T_8 - Bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha	4.37 (17.09)	5.39 (27.02)	86.55	73.10	5488	4.17 (15.43)	5.76 (31.23)	86.35	80.08	6232
T_9 - Bispyribac sodium 10% SC at 20 g a.i./ha	6.70 (42.91)	7.29 (51.15)	60.74	59.00	5167	5.95 (33.40)	8.17 (64.69)	67.02	61.76	5911
T_{10} - Metamifop 10% SE at 50 g a.i./ha	7.43 (53.20)	8.55 (71.14)	58.15	47.89	4276	6.49 (40.09)	8.03 (62.44)	63.28	55.67	4968
T_{11} - Hand weeding twice on 25 and 45 DAS	9.22 (82.93)	6.37 (38.54)	25.95	69.32	5256	9.21 (82.90)	6.84 (44.75)	5.36	73.56	5908
T_{12} - Unsprayed control	10.35 (105.02)	12.57 (156.13)	-	-	2734	8.77 (85.93)	11.61 (132.78)	-	-	3012
SEd	0.65	0.62	-	-	352	0.50	0.60	-	-	309
CD (P=0.05)	1.38	1.28	-	-	688	1.02	1.23	-	-	623

Figures in parenthesis are original values; Data subjected to square root transformation; DAHS: Days after herbicide spray

Table 3: Effect of weed management treatments on soil bacteria (x 10^6 CFU/g) and soil fungi (x 10^4 CFU/g) in direct seeded rice.

Treatments	Soil bacteria (x 10^6 CFU/g)				Soil fungi (x 10^4 CFU/g)			
	7 DAHS	15 DAHS	30 DAHS	At Harvest	7 DAHS	15 DAHS	30 DAHS	At Harvest
T_1 - Bispyribac sodium 4% SE + metamifop 10% SE at 42 g a.i./ha + wetter	14.83	16.23	17.96	18.63	4.08	4.32	4.87	4.96
T_2 - Bispyribac sodium 4% SE + metamifop 10% SE at 56 g a.i./ha + wetter	13.87	16.01	17.56	18.82	3.95	3.84	5.73	5.62
T_3 - Bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter	12.86	15.86	17.88	18.52	3.33	3.88	4.74	4.89
T_4 - Bispyribac sodium 4% SE + metamifop 10% SE at 140 g a.i./ha + wetter	10.56	14.88	16.92	17.88	2.82	3.56	5.62	4.80
T_5 - Almix (Chlorimuron + metsufuron 20% WP) at 4 g a.i./ha	12.62	16.21	17.09	18.11	3.62	3.75	4.96	5.08
T_6 - Clincher (Cyhalofop Buthyl 10% EC) at 80 g a.i./ha	11.56	16.25	17.13	18.01	2.98	3.60	4.56	4.96
T_7 - Bispyribac sodium 10% SC at 20 g a.i./ha + wetter	13.62	15.92	16.83	18.68	4.21	3.89	4.86	4.98
T_8 - Metamifop 10% SE at 50 g a.i./ha + wetter	11.72	16.85	17.22	17.32	3.66	4.22	5.42	4.86
T_9 - Bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha	12.64	16.23	17.11	18.42	3.96	4.01	4.52	4.88
T_{10} - Bispyribac sodium 10% SC at 20 g a.i./ha	11.86	16.85	17.73	18.78	4.09	3.98	4.21	4.96
T_{11} - Metamifop 10% SE at 50 g a.i./ha	10.03	15.68	16.16	18.55	4.68	4.22	4.56	4.72
T_{12} - Hand weeding twice on 25 and 45 DAS	16.58	17.22	17.86	18.24	4.98	4.85	5.02	5.16
T_{13} - Unsprayed control	17.16	17.82	18.56	18.94	4.74	4.99	5.12	5.58
S Ed	0.87	1.12	1.32	1.58	0.33	0.55	0.64	0.72
CD (P=0.05)	1.78	NS	NS	NS	0.67	1.12	NS	NS

DAHS: Days after herbicide spray

Table 4: Effect of weed management treatments on soil actinomycetes (x 10^2 CFU/g) and dehydrogenase (µg TPF released/g dry weight of soil) in direct seeded rice

Treatments	Soil actinomycetes (x 10^2 CFU g^{-1})				Dehydrogenase (µg TPF released/g dry weight of soil)
	7 DAHS	15 DAHS	30 DAHS	At Harvest	30 DAHS
T_1 - Bispyribac sodium 4% SE + metamifop 10% SE at 42 g a.i./ha + wetter	2.68	3.88	4.38	5.08	81.56
T_2 - Bispyribac sodium 4% SE + metamifop 10% SE at 56 g a.i./ha + wetter	2.32	3.60	4.56	4.98	77.16
T_3 - Bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter	2.24	3.28	4.66	5.02	72.54
T_4 - Bispyribac sodium 4% SE + metamifop 10% SE at 140 g a.i./ha + wetter	1.96	2.66	4.88	4.88	56.79
T_5 - Almix (Chlorimuron + metsufuron 20% WP) at 4 g a.i./ha	2.52	2.92	4.08	5.41	66.47
T_6 - Clincher (Cyhalofop Buthyl 10% EC) at 80 g a.i./ha	2.68	3.16	4.02	4.92	70.65
T_7 - Bispyribac sodium 10% SC at 20 g a.i./ha + wetter	2.74	3.24	4.22	5.23	74.96
T_8 - Metamifop 10% SE at 50 g a.i./ha + wetter	2.86	3.42	4.65	4.96	68.50
T_9 - Bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha	2.42	2.78	4.12	4.88	74.98
T_{10} - Bispyribac sodium 10% SC at 20 g a.i./ha	2.56	3.12	4.08	4.72	80.79
T_{11} - Metamifop 10% SE at 50 g a.i./ha	2.56	3.21	4.28	5.06	62.97
T_{12} - Hand weeding twice on 25 and 45 DAS	4.13	4.33	4.83	5.16	122.70
T_{13} - Unsprayed control	4.06	4.27	5.13	5.25	114.68
S Ed	0.29	0.36	0.44	0.58	1.22
CD (P=0.05)	0.21	NS	NS	NS	2.67

4. Conclusion
Pre-emergence herbicide application of bispyribac sodium 4% SE + metamifop 10% SE at 70 g a.i./ha + wetter at 100 ml/ha at all different doses obtained higher grain yield because of lower total weed density and weed biomass in these herbicide combination treated plots when compared to individual herbicide application. Microbial populations in the herbicide treated plots were more or less similar to the unsprayed control thus indicating that herbicides have no detrimental effect on soil health at the applied doses. The toxic effects of herbicides are normally most severe immediately after application. Later on, micro-organisms take part in a degradation process and then the degraded organic herbicides provide carbon rich substrates which in terms maximize the microbial population in the rhizosphere. Among different herbicide combination treated plots, POE application of bispyribac sodium 4% SE + metamifop 10% SE at 70, 56 and 42 g a.i./ha + wetter at 100 ml/ha recorded maximum dehydrogenase activities.

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