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Impact of Herbicide Combinations on Soil Microflora, Enzyme Activity, Nutrient Dynamics, and Weed Index in Dry Direct-Seeded Rice

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ABSTRACT

Dry direct-seeded rice (DSR) is gaining prominence as a sustainable alternative to conventional transplanting due to labor shortages and water scarcity. However, weed infestation remains a critical challenge in this method, significantly reducing yields. This study evaluates the impact of various herbicide combinations on soil microflora, enzyme activity, nutrient uptake, and weed control in DSR. A field experiment was conducted at the Main Research Station, Hebbal, University of Agricultural Sciences, Bengaluru, India, using a randomized block design with twelve treatments, including chemical and mechanical weed management methods. The results showed that herbicide combinations such as bensulfuron-methyl + pretilachlor followed by bispyribac sodium or triafamone + ethoxysulfuron effectively controlled weeds with minimal impact on soil microbial activity and soil enzymatic functions. These treatments enhanced nutrient uptake by the crop while reducing nutrient loss through weeds, leading to higher grain and straw yields. Hand weeding at 20, 40, and 60 days after sowing recorded the highest grain (5.50 t ha^{-1}) and straw yield (7.22 t ha^{-1}), which was statistically on par with bensulfuron-methyl + pretilachlor followed by bispyribac sodium (5.39 and 7.16 t ha^{-1}) and bensulfuron-methyl + pretilachlor followed by triafamone + ethoxysulfuron (5.29 and 7.03 t ha^{-1}). The lowest yields (grain: 1.40 t ha^{-1}, straw: 2.32 t ha^{-1}) were recorded in the weedy check.

Keywords: *Dry direct seeded rice, rice herbicides, bispyribac sodium, soil microflora*

Introduction

Rice is a vital staple food for millions across Asia countries, but its cultivation is highly water-intensive. Traditionally, rice is grown under submerged conditions, consuming significantly more water than other crops. In fact, irrigated paddy fields in Asia account for over 40% of the global freshwater used in agriculture [2]. The predominant method of rice establishment involves transplanting seedlings into puddled fields, which is a labor-intensive, time-consuming and water-demanding practice. However, in the present agricultural scenario labour shortages, rising labour costs, and increasing demand for irrigation water threaten the sustainability of this conventional system [23].

To overcome these challenges, dry direct-seeding of rice (DSR) has emerged as an alternative cultivation method. In this method, seeds are sown directly into dry fields, followed by incorporation through ploughing or harrowing. DSR offers potential benefits such as reduced water use, labor requirements by eliminating nursery raising, transplanting and also time saving, making it increasingly popular in countries like the Philippines, Malaysia, and Thailand [10] [15] .However, one of the major constraints of direct seeded rice is the heavy infestation of weeds, which can reduce grain yields by up to 100% if left unweeded [11].

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The simultaneous emergence of weeds and rice seeds, coupled with the shallow water depths maintained during the early growth stages, exacerbates weed competition.

Despite its benefits, the severe weed growth in dry direct-seeded rice remains a critical limiting factor in realizing its yield potential [15]. Weeds in this method compete with rice for essential resources such as nutrients, light, space, and moisture throughout the growing season, causing a more severe challenge compared to conventional transplanted rice. Hand weeding, though effective, is costly and labor-intensive, but labor availability is a significant constraint during peak periods of agricultural operations. Hence, chemical weed control is increasingly recommended as a cost-effective and labor-efficient solution for weed management [15] . On the other hand, several findings have reported that recommended doses of herbicides do not have a detrimental impact on the soil environment [16] . However, certain herbicides may affect non-target organisms, including microorganisms [8].

Considering all these challenges, an experiment was undertaken to evaluate the impact of various herbicide combinations on soil microflora, soil enzyme activity, nutrient uptake by the crop, nutrient removal by weeds, and the yield of dry direct-seeded rice.

Materials and Methods

The field experiment was conducted during *Kharif*, 2016 and 2017 at the Main Research Station, Hebbal, Bengaluru. The soil type was sandy loam with a pH of 6.8, with organic carbon of 0.55 percent. The experiment consisted 12 treatments, *viz.* bensulfuron methyl + pretilachlor*fb* triafamone + ethoxysulfurn (RM) (60 + 600 / 60 g/ha), oxadiargyl*fb*triafamone + ethoxysulfuron (RM) (100/ 60 g/ha), pendimetalin *fb*triafamone + ethoxysulfuron (RM) (1000/60 g/ha), pyrazosulfuron ethyl *fb*triafamone + ethoxysulfuron (RM) (20/60 g/ha), bensulfuron-methyl + pretilachlor*fb*bispyribac sodium (60 + 600/25 g/ha), oxadiargyl*fb*bispyribac sodium (100/25 g/ha), pendimethalin* *fb*bispyribac sodium (1000 /25 g/ha), pyrazosulfuron ethyl *fb*bispyribac sodium (20/25g/ha), pendimethalin* *fb*penoxsulam + cyhalofop butyl (RM) (1000 /135 g/ha), three mechanical weddings (20, 40, 60 DAS), hand weedings (20, 40, 60 DAS) and Weedy check (*Pendimethalin 38.7% CS) were tested in a Randomized Block Design with three replications.

Rice variety MAS 946-1 (Sharada) was sown with an inter row spacing of 30 centimeters and seeds were placed closely. The crop was fertilized with recommended dose of 100 kg N, 50 kg Pand 50 kg K/ha. The pre-emergence and post–emergence herbicides were applied using spray volume of 750 liters/ha and 500 liters/ha, respectively with Knap-sack sprayer having WFN nozzle.

The soil microbial population was estimated from soil samples collected at a depth of 0–15 cm. Rhizosphere soil samples were analyzed for total bacteria, fungi, and actinomycetes using the standard dilution plate count technique with specific nutrient media. Soil samples were thoroughly mixed and subjected to serial dilution (1 g soil in 100 ml distilled water), and enumeration of microorganisms was conducted using soil extract agar for bacteria, Martin's Rose Bengal agar with streptomycin sulphate for fungi, and Kusters' agar for actinomycetes. Colonies were counted, multiplied by the dilution factor, and expressed as colony-forming units (CFU) per gram of oven-dry soil. Soil urease activity was determined following Tabatabai's (1982) methodology, where unhydrolyzed urea was complexed with a coloring agent, and color intensity, proportional to urease, was measured at 510 nm using a UV spectrophotometer. Soil samples (1 g) were pre-incubated, treated with 0.2 ml toluene and 1 ml 0.2 M urea solution, incubated at 37°C for 2 hours, and extracted with KCl-AgSO4 for colorimetric analysis using Nessler's reagent. Soil dehydrogenase activity was determined where 2 g of soil was treated with 1 ml of 3% 2,3,5-triphenyl tetrazolium chloride and 2.5 ml distilled water under anaerobic conditions, incubated at 37°C for 24 hours, filtered, washed with methanol, and diluted to 100 ml, with red color intensity measured at 485 nm using methanol as a blank [3].

Plant and weed samples were collected at harvest, dried at 60°C in a hot air oven, powdered using a mixer grinder with stainless steel blades, and preserved in polythene covers for further analysis. Total nitrogen content was determined using the Kjeldahl method (Jackson, 1973), where 0.5 g of powdered sample was digested with concentrated H_2SO_4 in the presence of a digestion mixture, and NH_3 was distilled and titrated. For phosphorus and potassium analysis, 0.5 g of powdered samples were digested with concentrated HNO_3 followed by a di-acid mixture (HNO_3:$HClO_4$ in 10:4 ratio), with the final digest diluted to 100 ml. Phosphorus content was determined using the vanado-molybdo-phosphoric yellow color method, while potassium was estimated through a calibrated flame photometer. Nutrient uptake by paddy grain, straw, and weeds was calculated by multiplying nutrient concentration (%) with dry matter yield (kg ha⁻¹), using the formula:

$$\text{Nutrient Uptake (kg ha}^{-1}) = \frac{\text{Nutrient concentration (\%)} \times \text{Weight of dry matter (Kg ha}^{-1})}{100}$$

The grain and straw yield was recoded after the harvesting and threshing of the crop. The data collected was pooled for two years and was statistically analyzed using the standard procedure and the results were tested at five per cent level of significance [7]. The critical difference was used to compare treatment means.

Results and Discussion

Soil microflora and soil enzymes

The data pertaining to the soil microflora and soil enzymes is presented in table 1 and2. At 60 days after sowing significantly lowest population of bacteria (26.60 x 10^5 CFU g^{-1} soil), fungi (17.27 x 10^4 CFU g^{-1} soil), actinomycetes (14.60 x 10^4 CFU g^{-1} soil), urease (14.50 µg NH_4-N g^{-1} soil hr^{-1}) and dehydrogenase (83.92 µg TPF g^{-1} soil 24 hr^{-1}) activity was noticed in pre-emergence application of pendimethalin followed by penoxsulam + cyhalofop butyl as post-emergence compared to other herbicide combinations. Pre-emergence application of bensulfuron-methyl + pretilachlor followed by post-emergence bispyribac sodium as recorded bacteria (33.63 x 10^5 CFU g^{-1} soil), fungi (21.83 x 10^4 CFU g^{-1} soil), actinomycetes (18.46 x 10^4 CFU g^{-1} soil) population and also on soil enzymes like urease (18.33 µg NH_4-N g^{-1} soil hr^{-1}) and dehydrogenase (106.07 µg TPF g^{-1} soil 24 hr^{-1}) activity and bensulfuron methyl + pretilachlor followed by triafamone + ethoxysulfurnbacteria (33.55 x 10^5 CFU g^{-1} soil), fungi (21.78 x 10^4 CFU g^{-1} soil), actinomycetes (18.42 x 10^4 CFU g^{-1} soil) and also on soil enzymes like urease (18.27 µg NH_4-N g^{-1} soil hr^{-1}) and dehydrogenase (105.00 µg TPF g^{-1} soil 24 hr^{-1}) activity at 60 DAS. These herbicides are found to be effective in controlling weeds along least effect to soil biological activity. Similar results are observed after the harvest of the crop, significantly lowest population of bacteria (22.22 x 10^5 CFU g^{-1} soil), fungi (14.26 x 10^4 CFU g^{-1} soil), actinomycetes (10.98 x 10^4 CFU g^{-1} soil) and also on soil enzymes like urease (13.49 µg NH_4-N g^{-1} soil hr^{-1}) and dehydrogenase (73.06 µg TPF g^{-1} soil 24 hr^{-1}) activity was noticed in pre-emergence application of pendimethalin followed by penoxsulam + cyhalofop butyl as post-emergence. This reduction is very minor and other treatments have not found any detrimental effect on the soil microbial population. The best herbicide combinations which recorded significantly higher growth and yield have no detrimental effect on the soil microbial population and soil enzymes *i.e.* bensulfuron-methyl + pretilachlor fb bispyribac sodium bacteria (28.09 x 10^5 CFU g^{-1} soil), fungi (18.03 x 10^4 CFU g^{-1} soil), actinomycetes (13.88 x 10^4 CFU g^{-1} soil) and also on soil enzymes like urease (16.63 µg NH_4-N g^{-1} soil hr^{-1}) and dehydrogenase (90.24 µg TPF g^{-1} soil 24 hr^{-1}) activity and bensulfuron methyl + pretilachlor fbtriafamone + ethoxysulfurnbacteria (28.02 x 10^5 CFU g^{-1} soil), fungi (17.99 x 10^4 CFU g^{-1} soil), actinomycetes (13.84 x 10^4 CFU g^{-1} soil) and also on soil enzymes like urease (16.58 µg NH_4-N g^{-1} soil hr^{-1}) and dehydrogenase (89.33 µg TPF g^{-1} soil 24 hr^{-1}) activity after harvest[12] [19].

Nutrient uptake by crop and weeds

Adoption of different weed management practices significantly influenced the total nutrient uptake pattern of crop. Among the different weed management practices, hand weeding at 20, 40 and 60 days after sowing recorded significantly highest total nitrogen (86.88 kg ha^{-1}), phosphorus (33.93 kg ha^{-1}) and potassium uptake (70.39 kg ha^{-1}) compared to other treatments. However, it was statistically found on par with pre-emergence application of bensulfuron-methyl + pretilachlor followed by bispyribac sodium as post-emergence (84.71, 33.93 and 68.97 kg ha^{-1}, respectively) and application bensulfuron methyl + pretilachlor as pre-emergence followed bytriafamone + ethoxysulfurn (80.19, 31.32 and 64.97 kg ha^{-1}, respectively). Whereas the lowest nutrient uptake was observed in the weedy check (27.80, 9.68 and 24.80 kg ha^{-1}, respectively) due to sever competition by weeds. Higher nutrient uptake of crop in these treatments was mainly due to lower weed population and weed dry weight which helped the crop to grow well and absorb more nutrients from the soil and give less scope for loss of nutrients from soil through weeds [9]. who reported a significantly higher amount of NPK uptake with reduced competition by weeds shown in Table 3.

Hand weeding at 20, 40, and 60 DAS recorded significantly lowest total nitrogen (17.14 kg ha^{-1}), phosphorus (7.60 kg ha^{-1}), and potassium uptake (18.25 kg ha^{-1}) by weeds compared to other treatments. However, it was statistically found on par with pre-emergence application of bensulfuron-methyl + pretilachlor fb bispyribac sodium (18.14, 8.05 and 19.31 kg ha^{-1}, respectively) and application bensulfuron methyl + pretilachlor as pre-emergence fbtriafamone + ethoxysulfurn (18.49, 8.20 and 19.68 kg ha^{-1}, respectively). This is due effective suppression of weeds and better performance of crop in these treatments. These results are in conformation with the findings of [6] [11] and [9] which shown in Table 4.

Grain, straw yield, and weed index

Figure 1, illustrates the grain, straw yield, and weed index of dry direct-seeded rice among the different weed management practices.

Hand weeding at 20, 40, and 60 days after sowing recorded the highest grain (5.50 t ha^{-1}) and straw yield (7.22 t ha^{-1}), which was statistically on par with bensulfuron-methyl + pretilachlor followed by bispyribac sodium (5.39 and 7.16 t ha^{-1}) and bensulfuron-methyl + pretilachlor followed by triafamone + ethoxysulfuron (5.29 and 7.03 t ha^{-1}). The lowest yields (grain: 1.40 t ha^{-1}, straw: 2.32 t ha^{-1}) were recorded in the weedy check due to severe weed competition affecting growth and nutrient uptake. Among herbicide treatments, bensulfuron-methyl + pretilachlor followed by bispyribac sodium had the lowest weed index (2.11%), while the weedy check had the highest (74.49%) due to drastic yield reduction. Superior nutrient uptake by the crop, reduced nutrient removal by weeds, and enhanced soil microflora contributed to achieving higher grain and straw yields in dry direct-seeded rice similar results are observed by [1] [20] and [4].

Conclusion

Considering the current labor constraints and water scarcity, dry direct-seeded rice cultivation is a more suitable alternative to conventional transplanted paddy in puddled fields. Effective weed management in dry direct-seeded rice can be achieved using bensulfuron-methyl + pretilachlor followed by bispyribac sodium or triafamone + ethoxysulfuron, with no adverse effects on soil microflora or soil enzymes. These herbicide combinations also enhanced nutrient uptake by the crop, minimized nutrient removal by weeds and contributed to superior rice yields.

Table 1: Soil microbial population at 60 DAS and after harvest in dry direct seeded rice as influenced by different weed management practices (Pooled data of two years)

Treatments	Bacteria (x 10^5 CFU g^{-1} soil)	Fungi (x 10^4 CFU g^{-1} soil)	Actinomycetes (x 10^3 CFU g^{-1} soil)	Bacteria (x 10^5 CFU g^{-1} soil)	Fungi (x 10^4 CFU g^{-1} soil)	Actinomycetes (x 10^3 CFU g^{-1} soil)
	60 DAS			At harvest		
T_1: Bensulfuron methyl + pretilachlor*fb* triafamone + ethoxysulfurn (RM)	33.55	21.78	18.42	28.02	17.99	13.84
T_2: Oxadiagyl*fb*triafamone + ethoxysulfuron (RM)	31.54	20.47	17.31	26.34	16.91	13.01
T_3: Pendimetalin*fb*triafamone + ethoxysulfuron (RM)	31.95	20.74	17.54	26.69	17.13	13.19
T_4: Pyrazosulfuron ethyl *fb*triafamone + ethoxysulfuron (RM)	27.60	17.91	15.15	23.05	14.79	11.39
T_5: Bensulfuron-methyl + pretilachlor*fb*bispyribac sodium	33.63	21.83	18.46	28.09	18.03	13.88
T_6: Oxadiargyl*fb*bispyribac sodium	30.94	20.09	16.98	26.35	16.76	13.00
T_7: Pendimethalin* *fb*bispyribac sodium	32.86	21.33	18.03	27.44	17.61	13.56
T_8: Pyrazosulfuron ethyl *fb*bispyribac sodium	29.38	19.07	16.13	24.54	15.75	12.12
T_9: Pendimethalin* *fb*penoxsulam + cyhalofop butyl (RM)	26.60	17.27	14.60	22.22	14.26	10.98
T_{10}:Mechanical weedings	31.85	20.67	17.48	26.60	17.07	13.14
T_{11}:Hand weedings	33.92	22.16	18.68	28.58	18.47	14.42
T_{12}:Weedy check	30.96	20.10	17.00	26.20	16.77	13.04
S.Em±	0.92	0.63	0.49	0.86	0.50	0.53
CD(p=0.05)	2.69	1.86	1.45	2.52	1.48	1.55
Initial	33.65	21.86	18.17			

**Pendimethalin (38.7% CS), RM: Ready Mix, fb: Followed by Spray volume: 750 L ha^{-1} for pre-emergence and 500 L ha^{-1} for post-emergence herbicides*

Table 2: Urease and dehydrogenase activity at 60 days after sowing and at harvest in drydirect seeded rice as influenced by different weed management practices (Pooled data of two years)

Treatments	Urease activity (µg NH_4-N g $soil^{-1}$ hr^{-1})	Dehydrogenase activity (µg TPF g $soil^{-1}$ 24 hr^{-1})	Urease activity (µg NH_4-N g $soil^{-1}$ hr^{-1})	Dehydrogenase activity (µg TPF g $soil^{-1}$ 24 hr^{-1})
	60 DAS		At harvest	
T_1: Bensulfuron methyl + pretilachlor*fb* triafamone + ethoxysulfurn (RM)	18.27	105.00	16.58	89.33
T_2: Oxadiagyl*fb*triafamone + ethoxysulfuron (RM)	17.17	98.71	15.59	83.98
T_3: Pendimetalin*fb*triafamone + ethoxysulfuron (RM)	17.40	100.01	15.79	85.09
T_4: Pyrazosulfuron ethyl *fb*triafamone + ethoxysulfuron (RM)	15.03	86.36	13.95	75.48
T_5: Bensulfuron-methyl + pretilachlor*fb*bispyribac sodium	18.33	106.07	16.63	90.24
T_6: Oxadiargyl*fb*bispyribac sodium	16.93	97.60	15.31	83.87
T_7: Pendimethalin* *fb*bispyribac sodium	17.91	103.64	16.25	88.17
T_8: Pyrazosulfuron ethyl *fb*bispyribac sodium	16.01	92.67	14.70	79.84
T_9: Pendimethalin* *fb*penoxsulam + cyhalofop butyl (RM)	14.50	83.92	13.49	73.06
T_{10}:Mechanical weedings	17.36	100.45	15.75	86.62
T_{11}:Hand weedings	18.65	106.34	16.98	90.49
T_{12}:Weedy check	17.04	98.33	15.45	83.43
S.Em±	0.50	3.26	0.54	2.64
CD(p=0.05)	1.46	9.57	1.58	7.73
Initial	18.14	105.45		

**Pendimethalin (38.7% CS), RM: Ready Mix, fb: Followed by Spray volume: 750 L ha^{-1} for pre-emergence and 500 L ha^{-1} for post-emergence herbicides*

Table 3 :Total nutrient uptake (kg ha^{-1}) by dry direct seeded rice at harvest as influenced by different weed management practices (Pooled date of two years)

Treatments	Nitrogen	Phosphorus	Potassium
T_1: Bensulfuron methyl + pretilachlor*fb* triafamone + ethoxysulfurn (RM)	80.19	31.32	64.97
T_2: Oxadiagyl*fb*triafamone + ethoxysulfuron (RM)	56.45	22.05	46.04
T_3: Pendimetalin*fb*triafamone + ethoxysulfuron (RM)	55.20	21.56	45.26
T_4: Pyrazosulfuron ethyl *fb*triafamone + ethoxysulfuron (RM)	57.80	22.51	47.20
T_5: Bensulfuron-methyl + pretilachlor*fb*bispyribac sodium	84.71	33.06	68.71
T_6: Oxadiargyl*fb*bispyribac sodium	59.59	23.25	48.55
T_7: Pendimethalin* *fb*bispyribac sodium	60.93	23.78	49.65
T_8: Pyrazosulfuron ethyl *fb*bispyribac sodium	60.50	23.63	49.30
T_9: Pendimethalin* *fb*penoxsulam + cyhalofop butyl (RM)	51.58	20.06	42.22
T_{10}:Mechanical weedings	52.21	20.44	42.63
T_{11}:Hand weedings	86.88	33.93	70.39
T_{12}:Weedy check	27.80	9.68	24.80
S.Em±	2.17	1.00	1.92
CD(p=0.05)	6.36	2.95	5.62

**Pendimethalin (38.7% CS), RM: Ready Mix, fb: Followed by Spray volume: 750 L ha^{-1} for pre-emergence and 500 L ha^{-1} for post-emergence herbicides*

Table 4: Nutrient uptake (kg ha^{-1}) by weeds in dry direct seeded rice at harvest as influenced by different weed management practices (Pooled data of two years)

Treatments	Nitrogen	Phosphorus	Potassium
T_1: Bensulfuron methyl + pretilachlor*fb* triafamone + ethoxysulfurn (RM)	18.49	8.20	19.68
T_2: Oxadiagyl*fb*triafamone + ethoxysulfuron (RM)	38.46	17.06	40.94
T_3: Pendimetalin*fb*triafamone + ethoxysulfuron (RM)	42.52	18.86	45.26
T_4: Pyrazosulfuron ethyl *fb*triafamone + ethoxysulfuron (RM)	41.01	18.19	43.65
T_5: Bensulfuron-methyl + pretilachlor*fb*bispyribac sodium	18.14	8.05	19.31
T_6: Oxadiargyl*fb*bispyribac sodium	28.26	12.54	30.08
T_7: Pendimethalin* *fb*bispyribac sodium	33.06	14.66	35.19
T_8: Pyrazosulfuron ethyl *fb*bispyribac sodium	25.13	11.14	26.75
T_9: Pendimethalin* *fb*penoxsulam + cyhalofop butyl (RM)	47.70	21.15	50.77
T_{10}:Mechanical weedings	41.93	18.60	44.63
T_{11}:Hand weedings	17.14	7.60	18.25
T_{12}:Weedy check	68.51	30.39	72.93
S.Em±	1.38	0.60	1.56
CD(p=0.05)	4.06	1.75	4.58

**Pendimethalin (38.7% CS), RM: Ready Mix, fb: Followed by Spray volume: 750 L ha^{-1} for pre-emergence and 500 L ha^{-1} for post-emergence herbicides*

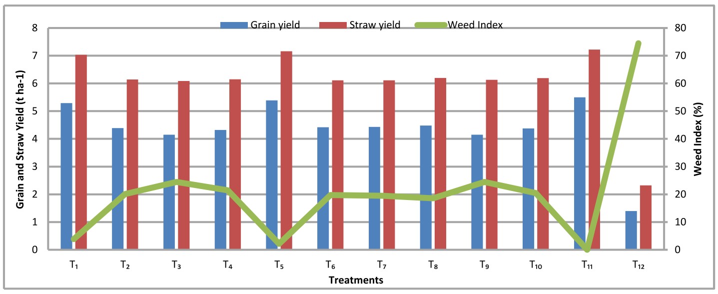

Figure 1: Grain yield, straw yield and weed index of dry direct seeded rice as influenced by different weed management practices (Pooled data of two years)

T_1:Bensulfuron methyl + pretilachlor*fb* triafamone + ethoxysulfurn (RM)
T_2:Oxadiagyl *fb*triafamone + ethoxysulfuron (RM)
T_3:Pendimetalin *fb*triafamone + ethoxysulfuron (RM)
T_4:Pyrazosulfuron ethyl *fb*triafamone + ethoxysulfuron (RM)
T_5:Bensulfuron-methyl + pretilachlor*fb*bispyribac sodium
T_6:Oxadiargyl *fb*bispyribac sodium
T_7:Pendimethalin* *fb*bispyribac sodium
T_8:Pyrazosulfuron ethyl *fb*bispyribac sodium
T_9:Pendimethalin* *fb*penoxsulam + cyhalofop butyl (RM)
T_{10}:Mechanical weedings
T_{11}:Hand weedings
T_{12}:Weedy check*Pendimethalin (38.7% CS), RM: Ready Mix, fb: Followed by Spray volume: 750 L ha^{-1} for pre-emergence and 500 L ha^{-1} for post-emergence herbicid

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