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Effect of Herbicide Application on Weed Density and Yield of Wet Direct Seeded Spring Rice at Sundarpur, Nepal

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ABSTRACT

Weeds are the major challenges for Direct Seeded Rice (DSR). A field experiment was carried out to evaluate the effect of herbicide application on weed density and yield of wet-direct seeded spring rice using Randomized Complete Block Design (RCBD), each treatment replicated thrice. The treatments consisted of three sole applications of pre-emergence herbicides (Pendimethalin @1000g a.i per ha, Pretilachlor @600g a.i ha^{-1} and Butachlor 1500 g a.i ha^{-1} at 3 DAS), six sequential applications of herbicides pre (at 3 DAS) and post emergence herbicides (at 22 DAS) (Pendimethalin *fb* Bispyibac Na @1000 *fb* 25 g a.i. ha^{-1}, Pretialchlor *fb* Bispyibac Na @ 600 *fb* 25 g a.i. ha^{-1}, Butachlor *fb* Bispyibac Na @ 1500 *fb* 25 g a.i. ha^{-1}, Pendimethalin *fb* 2,4-D EE @1000 *fb* 500 g a.i. ha^{-1}, Pretialchlor *fb* 2,4-D EE @600 *fb* 500 g a.i. ha^{-1}, Butachlor *fb* 2,4-D EE @1500 *fb* 500 g a.i. ha^{-1}) and two controls (weed free and weedy check). Result revealed that herbicide application significantly reduced the weed density (35.15-86.28%) compared with weedy check. Sequential application of pre and post emergence herbicides were more effective in reducing weed density and dry weight compared to sole application of herbicide. And among all sequential application, Pendimethalin *fb* Bispyribac sodium (119.3 m^{-2} and 124.33 m^{-2}) and Butachlor *fb* Bispyribac sodium (129.3 m^{-2} and 148.0 m^{-2}) were better and reduced weed density higher than other sequential application at 30 DAS and 90 DAS, whereas at 60 DAS, the highest reduction in total weed density was observed in Pretilachlor *fb* 2,4-D EE (90.0 m^{-2}) and Butachlor *fb* Bispyribac sodium (93.0 m^{-2}). The higher grain yield was observed in sequential application of pre-emergence herbicides followed byBispyribac sodium and was statistically similar with weed free plot. Up to 55.70% yield reduction was observed due to weeds. Therefore, sequential application of pre and post emergence herbicides: Pendimethalin *fb* Bispyribac sodium, Butachlor *fb* Bispyribac sodium and Pretilachlor *fb* Bispyribac sodium seems better in terms of reducing weed density and producing higher grain yield in wet-direct seeded spring rice.

Keywords: Weeds, herbicides, rice, density, yield

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INTRODUCTION

Rice is a staple food crop of Nepalese people (Gadal et al 2019). In Nepal, rice was cultivated in 1.47 million hectares of agricultural land with 5.62 million tons of production having 3.80t ha^{-1} productivity during 2020/21. About 92 % of rice area falls under main season while 7 percent is under spring season (CDD 2015). Rice cultivation during spring season results in higher productivity (4.4 t ha^{-1}) and efficient utilization of land and resources (Osti et al 2017). Rice in Nepal is planted mainly by two methods i.e. transplanting and direct seeding. Wet-DSR is being popular due to its higher water use productivity and energy use efficiencies, Dhakal et al 2019).

Weeds are the major constraint for the success of wet-DSR (Rao et al 2007, Singh 2008, Kamboj et al 2022). Emerging DSR seedlings are less competitive with weeds and the initial flushes of weeds is not controlled by flooding (Fujisaka et al 1993, Kumar et al 2008, Gyawaly et al 2021). Yield reductions due to weed competition are greater in wet-seeded than in transplanted rice but less than dry-DSR (Gopalakrishna 1977, Mabbayad and Moody 1985, Raj and Syriac 2017). Herbicidal weed control method is preferred to hand weeding because of its higher effectiveness and economics (Chauhan and Johnson 2011, Shah et al 2021).

Manual weeding is getting increasingly difficulty due to labour scarcity and rising wages rates. Chemical weed control may be a viable alternative to manual weeding as it costs less and demands less labour (Chauhan and Johnson 2011). It is now possible to improve yield of DSR by controlling weeds (Mishra and Singh 2008). The use of herbicides in rotational manner (pre-emergence herbicides followed by post-emergence) helps in controlling all types of weeds effectively rather than the use of single herbicide (Khaliq et al 2011).

Moreover, various herbicides are available in the market so that their effectiveness and economic viability is still unknown especially under wet direct seeded rice. Therefore, an experiment was done to evaluate the effect of herbicides application on weed density and yield of wet-direct seeded rice during spring season at Sundarpur, Kanchanpur.

MATERIALS AND METHODS

Location

Research was conducted at Sundarpur, Kanchanpur during the spring season of 2021 from March to July. It is located at 5 km Southeast from Mahendranagar, headquarter of Kanchanpur district. The experimental site is situated at 28º50’13” North latitude and 80º19’16” East longitudes with an elevation of 256 meters above mean sea level. During the crop growth duration, the average monthly maximum temperature ranged from 27^{o}C (March) to 35^{o}C (July) and average minimum temperature ranged from 9^{o}C (March) to 27^{o}C (July) (Fig.1). The total rainfall during experimental period was 666.86 mm and the lowest (35.06 mm) in April and the highest (280.69 mm) in July. The soil of experimental plot was neutral (7.4) with sandy loam texture. Soil organic matter was 3.82%, total nitrogen (0.12%) and available potassium 248 kg ha^{-1} were found medium. Available phosphorus was higher with 128.24 kg ha^{-1}.

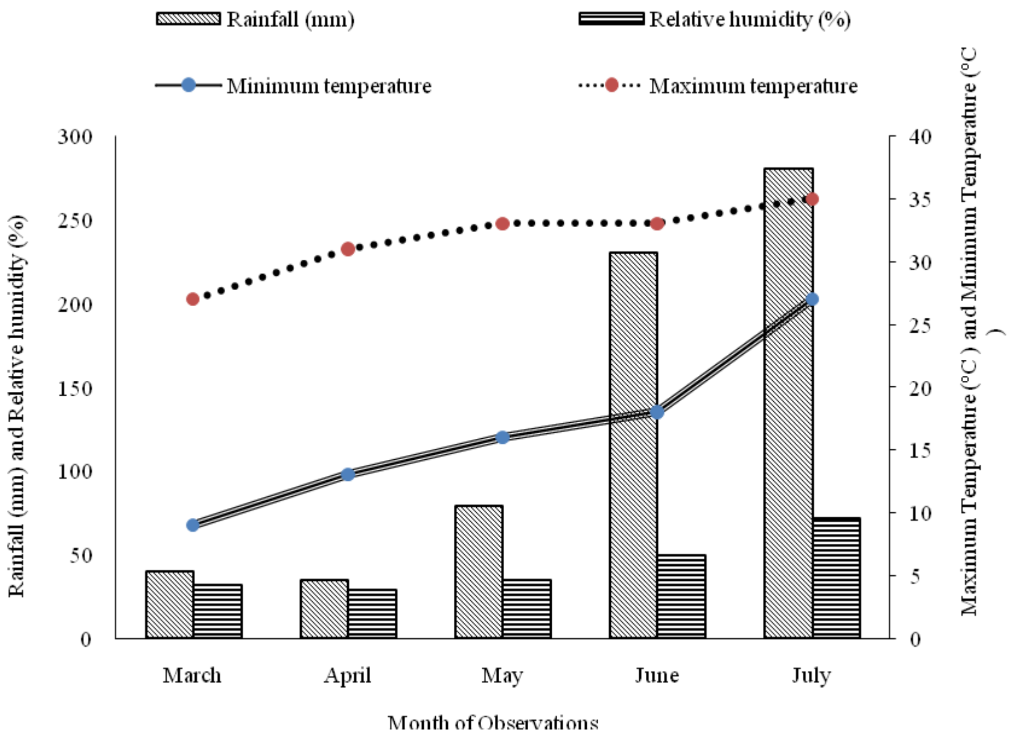

Figure 1. Weather condition during the course of experimentation at Sundarpur, Kanchanpur, Nepal from March to June, 2021

(Source: NASA POWER - https://power.larc.nasa.gov/data-access-viewer/)

Experimental design and treatments

The experiment was done by using Randomized Complete Block Design (RCBD) with eleven treatments replicated thrice.

Table 1. Treatment details of the experiment

Treatment ID	Treatment Details	Dose g a.i. ha^{-1}	Application time DAS
T_1	Pendimethalin	1000	3
T_2	Pretilachlor	600	3
T_3	Butachlor	1500	3
T_4	Pendimethalin *fb* Bispyribac-Na	1000 *fb* 25	3 *fb* 22
T_5	Pretilachlor *fb* Bispyribac-Na	600 *fb* 25	3 *fb* 22
T_6	Butachlor *fb* Bispyribac-Na	1500 *fb* 25	3 *fb* 22
T_7	Pendimethalin *fb* 2,4-D EE	1000 *fb* 500	3 *fb* 22
T_8	Pretilachlor *fb* 2,4-D EE	600 *fb* 500	3 *fb* 22
T_9	Butachlor *fb* 2,4-D EE	1500 *fb* 500	3 *fb* 22
T_{10}	Weedy Check	NA	NA
T_{11}	Weed free	NA	Weekly

Weeds were managed as per the treatments. Weedy check was left weedy and weed free was kept devoid of weeds throughout the growing period by hand weeding at 7 days up to panicle initiation stage. Respective herbicides were applied with the help of calibrated knap sack sprayer at specified dates with recommended doses.

Table 2. Herbicides used with their recommended dose, available form, and application time

Herbicides	Trade name	Dose a.i. ml/lit of water	Days of spray
Pendimethalin	Penda 30%EC	4ml	3 DAS
Pretilachlor	Sacfit 50% EC	2ml	3 DAS
Butachlor	Mahachlor 50% EC	3 ml	3 DAS
2,4-D Ethyl ester	Hit-44 38% EC	2.2ml	22 DAS
Bispyribac Na	Green Label 10% SC	0.5 ml	22 DAS
Weed free	-	Hand weeding	7 days interval

Crop Management

The size of the individual plot was 12 m^2 and net plot area of 6.4 m^2, consisting 15 rows with 20 cm row spacing and continuous sowing. The variety used was Hardinath-1 with the seed rate of 70 kg ha^{-1} (sprouted seed) under puddled condition. Chemical fertilizer were applied at 120:40:40 kg N: P_2O_5: K_2O kg ha^{-1} respectively. All other crop management practices were followed as per the recommendation.

Sampling and Measurements

Effective tillers were recorded from one full row of 4 m length of each plot. The row was selected randomly outside from the net plot except boarder rows and other data recorded rows. This was done just before harvesting and mean was calculated and the number was expressed per square meter. The total number of filled grains were counted from randomly selected 20 panicles just before harvesting the total unfilled, shriveled grains per panicle were also counted from the selected panicles and sterility percent was calculated. Thousand grains were taken from the grain yield of net plot and weighed with the help of portable automatic electronic balance at exact moisture content and mean was calculated and expressed in g at 14% moisture level. Biomass yield and grain yield was taken at harvesting from net plot having area of 6.4 m^2. Dicky Johns Multi-grain moisture meter was used to record the moisture percentage of the grain. Finally grain yield was adjusted at 14% moisture using the formula as suggested by Paudel (1995).

$$\text{Gain yield (kg/ha) at 14\% moisture} = \frac{(100\text{-MC}) \times \text{Plot yield (kg)} \times 10000\ (m^2)}{(100\text{-}14) \times \text{net plot area } (m^2)}$$

Where, MC is the moisture content in percentage of the grains.

Straw yield was obtained by deducting grain yield from total biomass yield of net plot. Sample of straw obtained was oven dried and expressed in 0% moisture content. Harvest index (HI) was computed by dividing grain yield at 0% moisture with the total dry matter yield (grain yield at 0% moisture and straw dry weight.

Weeds observed in experimental sites were identified and recorded with their categories. Weeds were categorized as a major weed if the number and dry biomass of the weed species is equal or more than the mean number and mean dry biomass. The major weed species was identified through the photos taken from the internet and weed catalogue were used for the identification of weeds. Number of weeds emerged were counted in area between 2nd and 3rd rows of rice i.e., from $0.1m^2$ (50cm × 20cm) at 30 DAS, 60 DAS, and 90 DAS. Total number of weeds was calculated by summation of total broadleaf weeds, narrow leaf weed and sedges. Thereafter total dry weight of weed samples of broadleaf weed, narrow leaf weed and sedges were worked out. Dry matter deposition was determined by oven drying weed samples at 70 ^{0}C for 72 hours.

Weed Index (WI) was calculated using the following formula given by Gill (1969).

$$\text{Weed Index (WI)} = \frac{X-Y}{X} x\ 100$$

Where,

X= Yield from minimum weed competition plot (weed free plot)

Y= Yield from the treatment for which WI is to be worked out (treated plot)

Statistical analysis

The data were subjected to analysis of variance, and Duncan‘s multiple range test at α level 0.05 (DMRT) for mean separations (Gomez and Gomez 1984). The data on weeds was transformed by square root transformation. MS Excel was usedfor the graphical representation and R-Studio for data analysis.

RESULTS AND DISCUSSION

Weed flora

The dominant weeds of broad leaves, grasses and sedges observed at different growth stages of wet direct seeded rice are listed in Table 3

Table 3. Description of weeds recorded at different growth stages of wet-direct seeded spring riceat Sundarpur, 2021

S.N.	Scientific name	Local name	Common name	Family	Habit
1.	*Amaranthus tuberculatus* (Moq.) Sauer	Latte	Rough fruit water hemp	Amaranthaceae	AH
2.	*Monochoria vaginalis* (Sw.) Willd.	Karkale jhar	Pickerel weed	Pontederiaceae	AH/PH
3.	*Caseulia axillaris* (Roxb.)	Thuke jhar	Pink-node grass	Asteraceae	AH
4.	*Marsilea polycarpa* Hook. And Grev.	-	Water clover	Oxalidaceae	PH
5.	*Ludwigia octavalvis* (Jacq.)	Bhulavangah	Mexican primrose willow	Onagraceae	PH
6.	*Centella asiatica* (L.) Urb	Godtapre	Spadeleaf	Apiaceae	PH
7.	*Eclipta prostrata* (L.)	Bhringraj	False daisy	Compositae	AH
8.	*Lindernia dubia* (L.) Pennell	-	Yellowseed false pimpernel	Linderniaceae	AH
9.	Polygonum aviculare (L.)	-	Pigweed	Polygonaceae	AH
10.	*Cynodon dactylon* (L.) Pers	Dubo	Bermuda grass	Poaceae	PH
11.	*Digitaria ciliaris* (Retz) Koeler	Banso	Crab grass	Poaceae	AH
12.	*Echinochloa crus-galli* (L.) P. Beauv.	Sawa	Barnyard grass	Poaceae	AH
13.	*Echinochloa colonum* (L.) Link	Sawa	Awnless barnyard grass	Poaceae	AH
14.	*Cyperus iria* (L.)	Mothe	Rice flat sedge	Cyperaceae	AH
15.	*Cyperus difformis* (L.)	Mothe	Variable flatsedge	Cyperaceae	AH
16	*Fimbristylis miliaceae* (L.) Vahl	Jwane	Globe fringerush	Cyperaceae	AH/PH

A, Annual, P, Perennial, H, Herb, D, Dicot, M, Monocot, S, Sedge

Weed density and weed dry weight

The weed density and dry weight at each observation was significantly influenced by both sole and sequential application of herbicides (Table 4 and 5). Weed density and dry weight was significantly lower in all herbicide applied plots as compared with weedy check. The highest reduction in weed density was in sequential application of Pendimethalin *fb* Bispyribac sodium at 30 DAS and 90 DAS but in Pretilachlor *fb* 2,4-D EE at 60 DAS. The highest reduction in weed dry weight was in sequential application of Pendimethalin *fb* Bispyribac sodium at 30 DAS and Pendimethalin *fb* 2,4-D EE at 60 DAS and 90 DAS as compared with weedy check.

This was due to the reason that sole application of pre-emergence herbicide kills the first flush of weed and the effect remain up to 10-15 days of application and after that application of post-emergence herbicide application kill the second flush of weed resulting in the lower density and dry weight in sequential application of herbicides as compared with sole application of herbicide. Sequential application of pre and post-emergence herbicides effectively controlled weeds by broadening the spectrum of weed control (Mahajan et al 2009). Similar result was reported by Marahatta et al (2017) and Marasini et al (2020). Bispyribac sodium was effective in killing all types of weeds so, Pendimethlain *fb* Bispyribac sodium showed lower weed densities in wet-DSR field. Goswami et al (2017) also recorded lower weed density and dry weight in Pendimethalin applied plots.

Table 4. Weed density (no. m^{-2}) of wet-direct seeded spring rice as influenced by herbicide application at Sundarpur, Kanchanpur, Nepal, 2021

Treatments	**Weed density**		
	30 DAS	**60 DAS**	**90 DAS**
Pendimethalin	14.20^{bc}(201.67)	15.51^{bc}(251.67)	15.56^{b}(241.67)
Pretilachlor	14.69^{b}(215.67)	15.76^{bc}(250.00)	15.79^{b}(249.00)
Butachlor	14.30^{bc}(204.33)	16.76^{b}(283.33)	15.83^{b}(250.67)
Pendimethalin *fb* Bispyibac Na	10.88^{d}(119.33)	10.55^{cd}(116.67)	11.03^{c}(124.33)
Pretilachlor *fb* Bispyibac Na	11.83^{bcd}(140.00)	12.20^{bcd}(156.67)	12.52^{bc}(156.67)
Butachlor *fb* Bispyibac Na	11.25^{cd}(129.33)	9.52^{d}(93.00)	12.06^{c}(148.00)
Pendimethalin *fb* 2,4-D EE	11.93^{bcd}(143.33)	9.52^{d}(110.00)	12.63^{bc}(161.67)
Pretilachlor *fb* 2,4-D EE	12.88^{bcd}(177.33)	9.36^{d}(90.00)	13.07^{bc}(180.67)
Butachlor *fb* 2,4-D EE	13.59^{bcd}(190.00)	11.99b^{cd}(146.67)	14.01^{bc}(200.00)
Weedy free	5.56^{e}(30.67)	5.15^{d}(25.00)	5.14^{d}(26.00)
Weed check	18.24^{a}(332.67)	23.87^{a}(583.33)	19.83^{a}(396.00)
SEm (±)	1.11	1.88	1.18
LSD (=0.05)	3.28	5.56	3.48
CV (%)	15.21	24.87	15.22
Grand mean	**12.67**	**13.12**	**13.40**

fb, followed by, DAS, days after sowing, SEm, standard error of mean, LSD, least significance difference, CV, coefficient of variation. Treatment mean *fb* same letter(s) are not-significantly different from each other within column based on DMRT at 0.05 level of significance, The figures in the parenthesis represent the original value and outside the parenthesis the square root transformation value ($\sqrt{}$(x+0.5)

Table 5.Dry weight (g m^{-2}) of weed biomass of wet-direct seeded spring rice as influenced by herbicide application at Sundarpur, Kanchanpur, Nepal, 2021

Treatments	Dry weight (g m^{-2}) of weed biomass		
	30 DAS	60 DAS	90 DAS
Pendimethalin	10.51^{bc}(110.80)	16.09^{cd}(258.86)	20.75^{cd}(430.97)
Pretilachlor	11.69^{b}(143.77)	24.57^{b}(610.44)	25.23^{b}(643.77)
Butachlor	11.05^{b} (122.00)	19.04^{c}(363.60)	22.36^{bc}(500.27)
Pendimethalin *fb* Bispyibac Na	5.71^{ef}(32.59)	10.64^{ef}(125.72)	15.85^{ef}(255.65)
Pretilachlor *fb* Bispyibac Na	7.71^{d}(60.32)	13.40d^{e}(182.83)	17.89^{de}(321.39)
Butachlor *fb* Bispyibac Na	7.24^{de}(53.57)	12.12d^{ef}(152.59)	14.89^{ef}(321.39)
Pendimethalin *fb* 2,4-D EE	8.28^{d}(68.86)	4.86^{gh}(31.42)	12.56^{f}(157.74)
Pretilachlor *fb* 2,4-D EE	8.79^{cd} (77.94)	10.73^{ef}(124.97)	14.60^{ef}(214.99)
Butachlor *fb* 2,4-D EE	8.53^{d}(72.94)	8.33^{fg}(41.33)	12.77^{f}(162.73)
Weedy free	4.14^{f}(17.28)	1.52^{h}(2.27)	3.91^{g}(15.59)
Weed check	14.36^{a} (207.47)	29.47^{a}(878.86)	31.67^{a}(1017.32)
SEm (±)	0.58	1.63	1.18
LSD (=0.05)	1.78	4.81	3.77
CV (%)	11.71	20.62	12.66
Grand mean	**8.91**	**3.22**	**17.49**

fb, followed by, DAS, days after sowing, SEm, standard error of mean, LSD, least significance difference, CV, coefficient of variation. Treatment mean *fb* same letter(s) are non-significance difference on Duncan multiple range test 05 level of significance, The figures in the parenthesis represent the original value and outside the parenthesis the square root transformation value (√(x+0.5)

Yield attributes

Effective tillers per square meter, number of grains per panicle, grain sterility percentage and grain yield were significantly influenced by herbicides application (Table 6).

Table 6.Yield and yield attributes as influenced by herbicide application in wet-direct seeded spring rice at Sundurpur, Kanchanpur, Nepal, 2021

Treatments	Yield attributes				
	Effective tiller m^{-2}	Grain panicle^{-1}	Sterility (%)	Thousand grain weight (g)	Grainyield (t ha^{-1})
Pendimethalin	273.33^{ab}	75.54^{f}	31.78^{b}	21.47	4.24^{efg}
Pretilachlor	265.00^{bc}	71.42^{f}	34.23^{ab}	20.93	3.69^{gh}
Butachlor	271.67^{ab}	73.88^{f}	32.50^{ab}	21.05	3.96^{fg}
Pendimethalin *fb* Bispyibac Na	311.67^{ab}	101.03^{b}	14.83^{gh}	22.74	6.22^{a}
Pretilachlor *fb* Bispyibac Na	296.67^{ab}	94.39^{c}	20.30^{ef}	22.17	5.61^{abc}
Butachlor *fb* Bispyibac Na	300.00^{ab}	96.92^{bc}	16.65^{fg}	22.41	5.95^{ab}
Pendimethalin *fb* 2,4-D EE	295.00^{ab}	92.14^{cd}	23.03^{de}	22.05	5.30^{bcd}
Pretilachlor *fb* 2,4-D EE	290.00^{ab}	81.68^{e}	27.33^{c}	21.79	4.70^{def}
Butachlor *fb* 2,4-D EE	293.33^{ab}	88.61^{d}	26.27^{cd}	21.92	5.01^{cde}
Weed free	320.00^{a}	110.6^{a}	11.04^{h}	23.33	6.39^{a}
Weedy check	221.67^{c}	64.64^{g}	36.28^{a}	19.90	2.82^{h}
SEm (±)	16.82	1.72	1.14	0.58	0.30
LSD (0.05)	49.63	5.09	3.87	NS	0.89
CV(%)	10.21	3.46	9.13	4.65	10.74
Grand mean	**285.30**	**86.39**	**24.93**	**21.80**	**4.90**

fb, followed by, DAS, days after sowing, SEm, standard error of mean, LSD, least significance difference, CV, coefficient of variation. Treatment mean *fb* same letter(s) within column are not-significantly different based on Duncan multiple range test 0.05 level of significance

Effective tillers were highest in Pendimethalin *fb* Bispyribac sodium which was statistically similar with all the herbicides treated plots and also with weed free plot. But Pretilachlor treated plot results in statistically similar effective tillers per square meter as compared with weedy check plot. Similarly, highest number of grains per panicle was observed in Pendimethalin *fb* Bispyribac sodium which was statistically similar with Butachlor *fb* Bispyribac sodium.Significantly, lowest grain sterility was observed in Pendimethalin *fb* Bispyribac sodium as compared with weedy check which was statistically similar with Butachlor *fb* Bispyribac sodium and also with weed free plot. Application of herbicides results in decreased in grain sterility as compared with weedy check except sole application of Pretilachlor and Butachlor.

The more number of panicle bearing tillers, grains per panicle might be attributed due to better nutrient acquisition, fertilization and translocation of photo assimilates under the influence of efficient weed control treatments (Hussain et al 2008, Mahajan et al 2009, Akbarran and Ali 2011, Pant and Sah 2020). These results are in line with those obtained by Mann et al (2008) who reported that sequential application of herbicides caused an increase in number of kernels per panicle in dry seeded rice due to better utilization of nutrient by crop.

Grain yield

Grain yield was significantly influenced by application of herbicides where highest grain yield was obtained in weed free plot and least in the weedy check plot. Herbicidal application results in significantly higher grain yield as compared with weedy check. Sequential application of Pendimethalin *fb* Bispyribac sodium resulted in highest grain yield which was statistically similar with Butachlor *fb* Bispyribac sodium and Pretilachlor *fb* Bispyribac sodium and also with weed free plot. The grain yield is actually the function of some yield attributing characters like effective tillers per meter square, grains per panicle, thousand grain weight etc. Mahajan and Timsina, (2011) also reported higher yield in Pendimethalin *fb* Bispyribac sodium treated plots. These results are parallel with Mann et al (2008),Khaliq et al (2011) and Karki et al (2022) who recorded maximum yield of rice in herbicide treated plots.

Weed Index

Weed index was significantly influenced by application of herbicides (Fig.1). The weed index was statistically higher in Pretilachlor treated plots and was statistically similar with weed check plot. Significantly lower weed index was obtained in Pendimethalin *fb* Bispyribac sodium and was statistically similar with Butachlor *fb* Bispyribac sodium, Pretilachlor*fb* Bispyribac sodium and Pendimeethalin *fb* 2,4-D EE.55.70% of yield reduction was observed due to the presence of weed. The lower yield loss was due to effective control of weeds. In some rice growing areas the infestation of both annual and perennial weeds can cause the yield losses of up to 50% (Tshewang et al 2016). Dangol et al (2020) found that reduction in yield by 67.90% was observed in wet-DSR due to weed infestation.

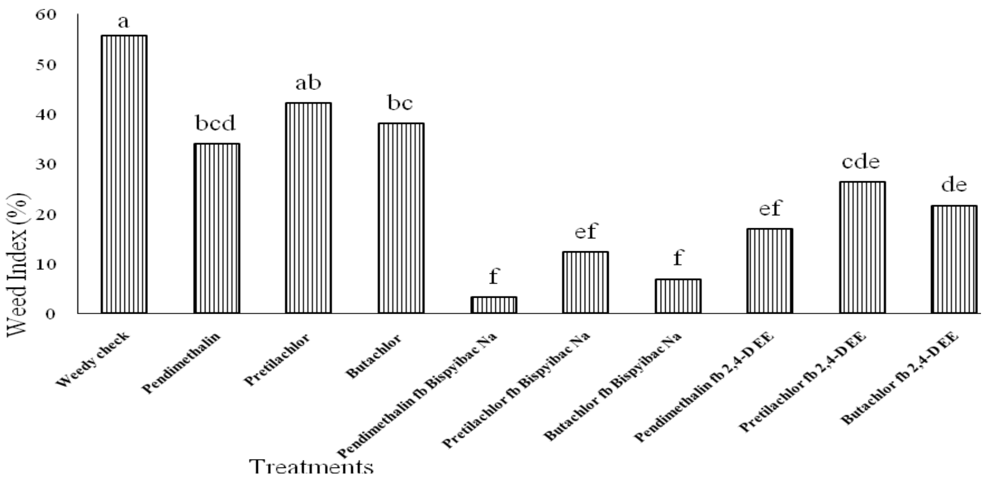

Figure 1. Weed index as influenced sole and sequential application of herbicides on wet-direct seeded spring rice at Sundarpur, Kanchanpur 2021

Mean separated by DMRT with same letter (s) are not significant at 5% level of significance

CONCLUSIONS

The sequential application of pre and post emergence herbicides: Pendimethalin *fb* Bispyibac Na @1000 *fb* 25 g a.i. ha^{-1}**,** Pretialchlor *fb* Bispyibac Na @600 *fb* 25 g a.i. ha^{-1}, Butachlor *fb* Bispyibac Na @1500 *fb* 25 g a.i. ha^{-1} at 3 and 22 DAS respectively seems better in terms of reducing weed density and producing higher grain yield in wet-direct seeded spring rice.

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AUTHORS' CONTRIBUTION

Suman Dhakal, Shrawan Kumar Sah, Suraj Singh Karkee designed the research plan & prepared the manuscript, and Chudamani Pant executed field experiment, generated necessary data, & critical revised and finalized the manuscript. Final form of manuscript was approved by all authors.

CONFLICTS OF INTEREST

The authors have no any conflict of interest to disclose.

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