



Halosulfuron + metribuzin effect on weed control in sugarcane and their carry over effect on succeeding lentil

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ABSTRACT

The study was conducted at GB. Pant University of Agriculture and Technology, Pantnagar during rainy seasons of 2012-13 and 2013-14 to evaluate the efficacy of halosulfuron 12% + metribuzin 55% WG ready-mix (RM) coded as CPL-1255 in sugarcane, and its carry over impact on succeeding lentil. The maximum weed suppression and the highest weed control efficiency were obtained with application of halosulfuron + metribuzin (RM) at higher doses (600 and 900 g/ha) applied as post-emergence, and were significantly better than the alone application of atrazine 50% WP and 2,4-D amine salt 50% SL at recommended doses. Combination of halosulfuron + metribuzin (RM) even applied at 600 g/ha was also found superior over atrazine 50% WP at 2000 g and 2,4-D amine 58% SL at 3500 g/ha while execution of twice hoeing (30 and 60 day after planting (DAP)) was also comparable to combination of halosulfuron + metribuzin at lower and higher doses at 450 and 900 g/ha, respectively, during 2012 and only to lower dose during 2013 with respect to cane yield. The ready mix application of halosulfuron + metribuzin at any dose did not pronounce any phytotoxic effect on sugarcane as well as carry over effect on the succeeding lentil in respect to germination, growth and grain yield. Overall microbial population was higher before application and decreased after application of herbicide at harvest. Herbicidal treated plots had no significant effect on bacterial counts. There was drastic reduction in microbial population at 7 day after application (DAA) of herbicide and increased thereafter. At harvest overall population of actinomycetes varied from 43.7% to 20.1% over weedy check.

Key words: Herbicide efficiency index, Phytotoxicity, Soil microbial population, Sugarcane, Yield

Sugarcane (*Saccharum officinarum*) is an important agro-industrial cash crop grown primarily for sugar production in India, and plays a pivotal role in agricultural and industrial economy of the country. Sugarcane is the most adaptable plant under varied ecological conditions. In tropical *Tarai* parts of Uttarakhand, weeds are the major threat in sugarcane production which affects the crop yields considerably. Singh *et al.* (2009) reported 20.3% yield gap in sugarcane because of the heavy infestation of weeds. The nature of weed problem in sugarcane cultivation is quite different from other field crops because sugarcane is planted with relatively wider row spacing and crop growth is very slow in the initial stages. It takes about 30 - 45 days to complete germination and next 60-75 days for developing full canopy cover (Anonymous 2001). Weed control is the most critical in early season prior to sugarcane canopy closure over the row middles. Crop-weed competition has been recorded to be 60-120 days after planting in spring cane and 150 days in autumn cane (Singh *et al.* 2011). In sugarcane 2-3 harrowings are required. However, due to labour

scarcity use at herbicide for weed management is gaining momentum. Several herbicides like atrazine, metribuzin and 2,4-D have been recommended. These herbicides effectively control only the specific weed species. Therefore, new herbicide combinations are required for broad-spectrum weed control in sugarcane.

Soil micro-organisms are an important link in soil-plant-herbicide-fauna-man relationships. Soil microbes are directly or indirectly affected by the impact of toxic substances of herbicides used to control in intensive agriculture. At normal recommended rates, herbicides of field are considered to have no major or long-term effect on microbial populations. It has been reported that some microorganisms were able to degrade the herbicide, while some others are adversely affected depending on the application rates and the type of herbicide used (Sebiomo *et al.* 2011). This soil microbial communities (like bacteria, fungi and actinomycetes) play critical role in litter decomposition and nutrient cycling, which in turn, affect soil fertility and plant growth (Chauhan *et al.* 2006, Tripathi *et al.* 2006, Pandey *et al.* 2007).

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Therefore, effects of herbicides on microbial growth, either stimulating or depressive, depend on the chemicals (type and concentration), microbial species weeds to be evaluated and environmental conditions (Zain *et al.* 2013). The study was aimed to evaluate the effect of commonly used herbicides on growth, yield and populations in soil microcosms from sugarcane crop.

MATERIALS AND METHODS

During first year (March, 2012 to January, 2013) and during second year (March, 2013 to January, 2014), the total annual rainfall received was 960 and 1759 mm and the relative humidity ranged from 18 to 97% and 23 to 97%, respectively. The maximum and minimum temperature ranged from 42.5 °C to 5.8 °C and 39.2 °C to 5.9 °C, respectively during first and second season. During succeeding crop growing season of both the year from January to March, 2013 and from February to March, 2014, the total rainfall 153.9 and 216.8 mm was received and the relative humidity ranged from 23 to 94% and 19 to 95%, respectively. The average maximum and minimum temperature were 38.9 °C and 9.5 °C and 37.5 and 7.0 °C during both the season, respectively.

The site of field experiment was at Norman E. Borlaug, Crop Research Center of G. B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar, Uttarakhand, India (28.97 °N, 79.41°E and elevation 243.8 m above mean sea level. The experiment was conducted during 2012-13 and 2013-14 to evaluate the efficacy of various doses of halosulfuron + metribuzin and its phytotoxicity, microbes in soil of sugarcane crop and carry over studies in succeeding lentil crop. The mean values of the soil of experimental field were neutral in reaction, electrical conductivity 0.37 dS/m having 66% silt, 18% clay and 16% sand in the experimental soil. The soil of experimental field was silt loam in texture and low in nitrogen, medium in phosphors and potassium. Organic carbon content was slightly high *i.e.* 0.78%.

The field experiment was laid out in randomized block design in triplicates comprised of eight treatments of halosulfuron + metribuzin at 300, 450, 600 and 900 g/ha, another standard check atrazine 2000 g/ha, 2,4-D amine salt 3500 g/ha, twice hoeing 30 and 60 day after planting (DAP) and weedy check.

The sugarcane variety 'COP 90223' was planted under irrigated ecosystem at spacing of 60 cm during 2012 and 2013. Atrazine 50% WP was applied as pre-emergence just after planting of cane sets, whereas, combination of halosulfuron + metribuzin (RM) and alone application of 2,4-D amine

salt were used as post-emergence with help of knapsack sprayer fitted with flat fan nozzle at a spray volume of water 500 l/ha. All the agronomic management practices were followed as per the standard recommendations.

Data on weed flora were recorded at 60 day after application (DAA) by placing a quadrat of 0.25 m² at four randomly selected places in all the plots of the experimental field and expressed in per m². The removed weed flora samples for weed count were kept in brown paper bags and oven dried at 70 °C for 72 hrs and expressed in g/m² and weed control efficiency (WCE) and herbicide efficiency index (HEI) were calculated by using standard formula. Yield was calculated on the basis of net plot and expressed in t/ha.

Soil samples (0-15 cm depth) from each experimental plot were collected randomly from four different places with the help of trowel and mixed them thoroughly to make the representative composite sample. The samples were collected for analysis on different stage, first sampling was on before application, and remain on different interval after the application of herbicide at 7, 15, 30, 60 days and at harvest stage. Approximately 150 g soil sample stored at 4 °C in deep freezer until analysis for microbial studies and rest part of the samples were air dried, processed and sieved through 2 mm sieve and analyzed for basic soil microbial properties. Two year average microbial population of the soil before application of herbicide, *viz.* total bacteria, actinomycetes and fungi were 16.43 x 10⁷ cfu/g, 17.43 x 10⁵ cfu/g and 14.24 x 10⁴ cfu/g, respectively.

The enumeration of the microbial population was done on agar plates containing appropriate media following serial dilution technique and pour plate method (Pramer and Schmidt 1965). Soil samples collected were analyzed by following serial dilution plating procedures. Soil extract agar medium (Lochhead and Chase 1943) was used for the count of total bacteria in soil. The population of fungi was estimated on Martin's Rose Bengal agar medium with streptomycin and Rose Bengal as dye in medium (Martin 1950). Actinomycetes population was counted by using Kenknight and Munaier's Medium agar medium (Chhonkar *et al.* 2002). After allowing for development of discrete microbial population during incubation at 28±2 °C in incubator, the colonies were counted and number of viable counts expressed as CFU/gram dry weight basis of soil. An incubation period of 48 h for bacteria, 48–72 h for fungi and one week for actinomycetes. Microbial population in the soil was computed by taking into account the soil dilutions.

For residual carry over study, the original experimental layout was kept undisturbed, and lentil was sown across the plots. The crop was raised as per recommended package and practices of lentil crop. Carry over effect of herbicides were studied in succeeding lentil crop in terms of plant population, pods/plant, number of grains/pod, 1000 grain weight and grain yield (t/ha).

To see the phytotoxicity of different treatments on crop, visual observations were recorded on chlorosis, necrosis, stunting, scorching, hyponasty and epinasty *etc.* on the prescribed 0-10 scale at 1, 3, 5, 7, 10, 15 and 30 days after herbicidal application in sugarcane.

The data were subjected to statistical analysis by analysis of variance method. All the data obtained from experiment were subjected to statistical analysis as per the method detailed by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Weed density

During both the seasons, major weed flora of the experimental field at 60 DAA consisted of grassy weeds, *viz.* *Echinochloa colona* (7.9 and 4.0%), *Eleusine indica* (6.4 and 12.2%), *Dactyloctenium aegyptium* (8.4 and 8.1%) and *Digitaria sanguinalis* (10.8 and 10.1%); broad-leaved weeds, (BLWs), *viz.* *Commelina benghalensis* (2.8 and 3.6%) and *Cleome viscosa* (1.6 and 3.0%), whereas, *Cyperus rotundus* (62.1 and 58.9%) was the only sedge.

Combination of halosulfuron + metribuzin (RM) applied as post-emergence at 600 and 900 g/ha and 2,4-D-amine salt applied at 3500 g/ha were the most effective against BLWs, and recorded 100% weed suppression as compared to other herbicidal treatments except *Cleome viscosa*. Whereas, two

hoeing (30 and 60 DAS) were effective in complete removal of BLWs in year 2012 only. Among the grassy weeds, similar observation was recorded by ready mix (RM) combination of halosulfuron + metribuzin at 600 and 900 g/ha in total elimination of *Echinochloa colona* during 2013. The application of lower dose of halosulfuron + metribuzin at 300 g/ha and 2,4-D amine salt at 3500 g/ha was at par with weedy check in reducing the population of *Echinochloa colona*, *Dactyloctenium aegyptium* and *Digitaria sanguinalis*.

Weed dry matter accumulation and weed control efficiency (WCE)

The mean data on dry matter of grassy weed at 60 DAA revealed that minimum accumulation (5.4 and 6.5 g/m²) and maximum weed control efficiency (71.0 and 81.1%) was obtained with ready mix application of halosulfuron + metribuzin at (900 g/ha) during 2012 and 2013, respectively, being at par with (600 g/ha), and significantly superior to rest of the treatments. Complete control of BLWs, was attained by the combined application of halosulfuron + metribuzin at 900 g/ha and 2,4-D amine at 3500 g/ha. The dry-matter accumulation of sedges (1.1 and 1.5 g/m²) was minimum with the highest dose of halosulfuron + metribuzin at 900 g/ha followed by 600 g/ha (**Table 1** and **2**). The reduction in dry-matter by application of halosulfuron + metribuzin at (900 g/ha) might be due to lower weed density. The effect of halosulfuron + metribuzin at (900 g/ha) was more pronounced as compared to atrazine in respect to non-grassy (BLWs and sedges) weeds in reducing the dry matter accumulation.

Herbicide efficiency index

Herbicide efficiency index (HEI) of different herbicides in each plot of the experiment was recorded at 60 DAA by addition of dry-matter

Table 1. Effect of different treatments on weed dry weight at 60 DAA during 2012 and 2013

Treatment	Dose (g/ha)	Total weed dry weight (g/m ²)					
		Grasses		BLWs		Sedges	
		2012	2013	2012	2013	2012	2013
Halosulfuron + metribuzin	300	7.8(60.1)	11.4(128.1)	1.9(2.6)	2.9(7.9)	1.4(1.2)	2.1(3.3)
Halosulfuron + metribuzin	450	6.6(42.5)	7.3(53.1)	1.2(0.4)	1.9(2.7)	1.3(0.7)	1.7(1.7)
Halosulfuron + metribuzin	600	6.1(36.6)	6.7(43.3)	1.1(0.2)	1.5(1.5)	1.2(0.5)	1.6(1.5)
Halosulfuron + metribuzin	900	5.4(28.5)	6.5(41.1)	1.0(0.0)	1.0(0.0)	1.1(0.3)	1.5(1.1)
Atrazine 50% WP (standard check)	2000	7.3(53.0)	9.8(95.4)	2.4(4.9)	3.6(12.2)	4.0(15.3)	13.9(14.7)
2,4-D Amine salt 58% SL (standard check)	3500	9.7(93.7)	12.7(161.2)	1.0(0.0)	1.0(0.0)	1.7(1.8)	1.5(1.3)
Hoeing twice	30 & 60 DAP	7.9(61.2)	9.7(93.5)	1.0(0.0)	2.2(4.5)	2.2(3.9)	2.3(4.1)
Untreated	-	10.0(98.3)	14.8(217.3)	2.7(6.2)	4.4(18.3)	4.2(16.3)	4.1(15.5)
LSD (p=0.05)		0.74	0.7	0.25	0.7	0.21	0.3

Values in parentheses were original and transformed to square root ($\sqrt{x+1}$) for analysis, DAP- Days after transplanting, DAA- Days after herbicidal application

accumulation of total grassy and non-grassy weeds (Table 2). HEI which indicates weed killing potential and phytotoxicity on the crop was the highest under combined application of halosulfuron + metribuzin at 900 g/ha followed by 600 g/ha. However, the application of 2,4-D amine salt at 3500 g/ha resulted in the lowest HEI.

Study on microbial population

Overall microbial population was high before herbicide application and decreased after application even upto harvest of the crop. Decline of the overall soil microbial population was more pronounced and drastic up to 7 DAA and then increased. Buildup of the micro flora was observed up to harvest of the crop.

Bacterial population (10⁷ cfu/g)

Herbicidal treated plots had no significant effect on bacterial counts. Soil bacterial populations in the control plot was higher than those of herbicide treated plots. The bacterial populations for all soil samples increased after 7 to 60 DAA. After the 7 to 60 DAA, the bacterial population varied from 6.51 x 10⁷ cfu/g to

12.59 x 10⁷ cfu/g. Drastic reduction was observed in microbial population at 7 DAA of herbicide (Figure 1A).

Actinomycetes population (10⁵ cfu/g)

The population of actinomycetes varied from 3.0 x 10⁷ cfu/g to 13.0 x 10⁷ cfu/g after 7 to 60 DAA of herbicide. At harvest, overall population of actinomycetes varied from 43.7 to 20.1% over weedy check (Figure 1B).

Fungal population (10⁴ cfu/g)

The fungal population fluctuated between the first and second week, while the control samples had the highest fungal population. The lowest population of fungus (3.61 x 10⁵ cfu/g) at 7 DAA was obtained with application of halosulfuron + metribuzin at 450 g/ha and increased by 70.2% after harvest of crop. It's evident from the results that up to 15 DAA, the herbicide had adverse effect on population of fungi in rhizosphere region. This indicates that the population of fungi started to increase from 30 DAA. Herbicidal application gave higher population (70.2 to 78.3%) of fungi from 7 DAA to harvest of crop (Figure 1C).

Table 2. Effect of different treatments on weed control efficiency (%) and herbicide efficiency index (%) at 60 DAA during 2012 and 2013

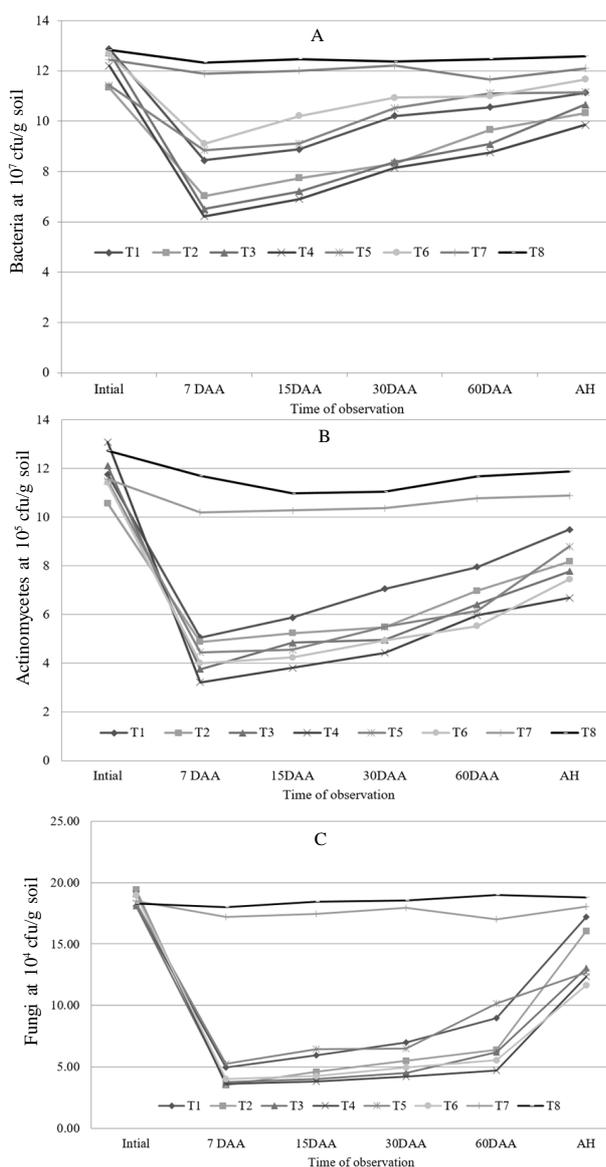
Treatment	Dose (g/ha)	Weed control efficiency (%)						Herbicide efficiency index (%)	
		Grasses		BLWs		Sedges			
		2012	2013	2012	2013	2012	2013	2012	2013
Halosulfuron + metribuzin	300	38.9	41.0	67.7	56.8	92.6	78.7	1.13	1.19
Halosulfuron + metribuzin	450	56.8	75.6	93.5	85.2	95.7	89.0	3.34	5.13
Halosulfuron + metribuzin	600	62.8	80.1	96.8	91.8	96.9	90.3	4.04	6.69
Halosulfuron + metribuzin	900	71.0	81.1	100.0	100.0	98.2	92.9	5.04	7.15
Atrazine 50% WP (standard check)	2000	46.1	56.1	20.9	33.3	6.1	5.1	1.29	1.11
2,4-D Amine salt 58% SL (standard check)	3500	4.7	25.8	100.0	100.0	88.9	91.6	0.47	0.23
Hoing twice	30 & 60 DAP	37.7	56.9	100.0	75.4	76.1	73.5	1.39	1.14
Untreated	-	-	-	-	-	-	-	-	-
LSD (p=0.05)		-	-	-	-	-	-	-	-

DAP- Days after planting, BLWs- Broad-leaf weeds, DAA- Days after herbicidal application

Table 3. Effect of different treatments on cane yield attributes of sugarcane during 2012 and 2013

Treatment	Dose (g/ha)	Milliable cane (t/ha)		Cane length (m)		Cane girth (cm)		Per cane weight (g)		Cane yield (t/ha)		Percent increase in yield over weedy	
		2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013
		Halosulfuron + metribuzin	300	71.4	86.4	1.9	2.1	7.4	7.2	873	817	63.5	71.1
Halosulfuron + metribuzin	450	89.9	90.8	2.0	2.2	7.8	7.4	990	1045	87.5	92.6	120.4	116.9
Halosulfuron + metribuzin	600	90.3	93.8	2.0	2.2	7.8	7.4	1000	1053	89.3	98.9	124.9	131.6
Halosulfuron + metribuzin	900	87.2	91.3	2.0	2.1	7.7	7.3	1003	1017	87.3	90.7	119.9	112.4
Atrazine 50% WP (standard. check)	2000	70.9	65.9	2.0	2.1	7.8	7.4	1000	1015	70.7	65.8	78.1	54.1
2,4-D Amine salt 58% SL (standard check)	3500	61.4	53.3	1.9	2.0	7.5	7.0	887	900	54.6	49.2	37.5	15.2
Hoing twice	30&60DAP	70.5	64.3	1.9	2.0	7.9	7.3	988	947	69.5	62.5	75.1	46.4
Untreated	-	49.2	46.8	1.6	1.9	7.1	6.9	807	820	39.7	42.7	-	-
LSD (p=0.05)		6.8	6.3	0.09	0.14	0.4	0.3	6.8	106.0	66.3	80.9	-	-

DAP- Days after planting



T¹ - Halosulfuron + metribuzin, T² - Halosulfuron + metribuzin, T³ - Halosulfuron + metribuzin, T⁴ - Halosulfuron + metribuzin, T⁵ - Atrazine (standard. check), T⁶ - 2,4-D Amine salt (standard check), T⁷ - Hoeing (2), T⁸ - Untreated

Figure 1. Effect of halosulfuron + metribuzin herbicide on soil bacterial (A), actinomycetes (B) and fungal (C) population at diferent intervals

Yield attributes and yield

Yield attributes of sugarcane varied significantly with various weed control treatments except cane length which did not show any significance over the herbicidal application (Table 3). During both the years maximum number of milliable cane ('000/ha) were recorded with ready mix application of halosulfuron + metribuzin at 600 g/ha, which was at par with 450 and 900 g/ha. Application of halosulfuron + metribuzin at 450 and 600 g/ha and atrazine (standard check) at 2000 g/ha was found to be similar for cane girth. Maximum cane weight (1003 g/cane) was recorded with application of halosulfuron + metribuzin at 900 g/ha and was at par with 600 g/ha during 2012. Whereas, during 2013, maximum cane weight (1053 g/cane) was obtained with application of halosulfuron + metribuzin 600 g/ha and was significantly superior to only lower dose applied at 300 g/ha. The yield parameters like milliable cane, cane length, cane girth, cane weight were significantly influenced by weed control treatments over the weedy check.

Maximum cane yield (89.3 and 98.9 t/ha) was recorded under halosulfuron + metribuzin applied at 600 g/ha. The effective control of weeds at early stage of growth might have resulted in better growth and yield of sugarcane. These results were in coincidence with findings of Singh *et al.* (2011). Application of 2,4-D amine salt at 3500 g/ha recorded significantly lower cane yield than other herbicidal treatments as well as twice hoeing (30 and 60 DAP).

Phytotoxic effect

During both the years, no phytotoxic effect in terms of scorching, necrosis, hyponasty and epinasty were seen in sugarcane crop with the application of halosulfuron + metribuzin at different doses. However, moderate to slight chlorosis and stunting was noted up to 3 DAA at highest dose of halosulfuron + metribuzin, which itself recovered and no phytotoxicity symptoms were observed thereafter

Table 4. Effect of different treatments on succeeding lentil during 2012 and 2013

Treatment	Dose (g/ha)	Plant (no./m ²)				Pods/plant		Grain/pod		1000 seed weight (g)		Grain yield (t/ha)	
		15 DAS		At harvest		2012-13	2013-14	2012-13	2013-14	2012-13	2013-14	2012-13	2013-14
		2012-13	2013-14	2012-13	2013-14								
Halosulfuron + metribuzin	300	105	98.2	92	84.0	46.0	42.8	1.60	1.50	21.6	21.1	1.45	1.12
Halosulfuron + metribuzin	450	107	97.5	93	82.5	46.2	43.0	1.62	1.48	21.7	21.0	1.47	1.07
Halosulfuron + metribuzin	600	106	98.0	93	83.2	46.1	43.4	1.61	1.51	22.0	21.2	1.39	1.15
Halosulfuron + metribuzin	900	107	101.0	94	83.5	46.5	42.8	1.59	1.49	22.2	21.5	1.42	1.10
Untreated		108	101.3	89	83.7	46.3	42.3	1.60	1.50	21.8	21.3	1.42	1.07
LSD (p=0.05)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

at 5, 7, 10, 15 and 30 DAA. Test herbicides did not show any phytotoxicity effect even at higher doses. Hence, there was no reduction in yield parameters due to phytotoxicity. Etheredge *et al.* (2010) also did not observe any reduction in sugarcane growth later in the growing season and any injury to the crop due to halosulfuron.

Residual effect of halosulfuron + metribuzin applied in sugarcane on succeeding lentil crop

The residual effect of treatments (applied to sugarcane) was not well pronounced on germination per cent and yield of lentil during both the years. All the yield and yield attributing characters were not significantly influenced at all the doses of halosulfuron + metribuzin and they were almost similar in all the treatments, including weedy check plots. Post-emergence application of halosulfuron + metribuzin against weeds in sugarcane crop during both the years was safe for growing lentil. These results were in coincidence with the findings of Chand *et al.* (2014) who also reported that none of the doses of halosulfuron affect the growth and yield of succeeding lentil crop. The combination of halosulfuron + metribuzin applied as post-emergence in sugarcane crop during both the years didn't cause any adverse effect on grain yield of succeeding lentil crop as yield achieved in treated plot was similar to the untreated plot. Therefore, application of halosulfuron + metribuzin against weeds in sugarcane was safe for growing succeeding lentil in winter season (**Table 4**).

Application of halosulfuron + metribuzin at 600 g/ha achieved maximum yield of sugarcane. Ready mix combination of halosulfuron + metribuzin was found more effective in reducing the population of BLWs and sedges as compared to grasses, without any phytotoxic effect on sugarcane and succeeding lentil. The bacterial population was more sensitive to herbicide application than fungi and actinomycetes.

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