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RESEARCH ARTICLE

Seed production potential of *Medicago denticulata* in relation to growth stage at the time of herbicide application

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ABSTRACT

Medicago denticulata Willd. is a winter annual weed infesting wheat. The present study was aimed to evaluate the effect of weed growth stage at the time of herbicide application (2,4-D, carfentrazone-ethyl and pre-mix herbicide metsulfuron-methyl plus sulfosulfuron) on efficacy of different post-emergence herbicides and herbicide carryover effects onto future generations. Herbicide sprays done at four-leaf stage of *M. denticulata* provided effective control whereas delayed application resulted in poor control with no visual injury. Significant increase in weed density was observed as the herbicides were sprayed at advanced growth stages of *M. denticulata*. Delayed application of all the herbicides at eight and twelve-leaf stage caused decrease in weed control efficiency than herbicide sprays done at four-leaf stage. However, application of herbicides at eight and twelve-leaf stages caused significant reduction in seed production potential of *M. denticulata* as compared to herbicide sprays done at four-leaf stage. Carfentrazone-ethyl had more pronounced effect on seed production potential of *M. denticulata* than 2,4-D. Whereas, metsulfuron-methyl plus sulfosulfuron was least effective in reducing fruit and seed number of *M. denticulata.* Seeds produced by *M. denticulata* plants after herbicide exposure were viable but dormant and exhibited decreased germination.

Keywords: Germination, Herbicide, Seed heteromorphism, Weed control efficiency

INTRODUCTION

M. denticulata is a winter annual weed native to Mediterranean basin but has also infested western and central Asia (Graziano *et al.* 2010). In India, this weed has invaded many states, *viz.* Punjab, Haryana, Jharkhand, Bihar, Madhya Pradesh and West Bengal. Among various dicotyledonous weeds, *M. denticulata* is the major problematic weed prevalent in wheat fields of Punjab (Kaur *et al.* 2015). The fruit (bur) is a prickly, flattened and coiled pod containing 3-5 kidney shaped seeds (Walsh *et al.* 2013).

Major herbicides used in India for control of dicotyledonous weeds in wheat are metsulfuron, 2,4-D and carfentrazone (Chhokar *et al.* 2015). Although herbicides provide cost-effective weed control but over-reliance on herbicides with a similar mode of action can rapidly lead to evolution of herbicide resistance in weeds (Bhullar *et al.* 2017). Weed growth stage at the time of herbicide application strongly influences the uptake, translocation and metabolism of herbicides. Herbicides applied at advanced weed growth stage increase the rate of herbicide degradation resulting in decreased herbicide efficacy (Singh and Singh 2004). Annual weeds mainly rely on renewable seed production to ensure their persistence; therefore, spraying herbicides at or near flowering can be used as an alternate approach for managing weeds by cutting down their seed production potential. Application of selective herbicides to dicotyledonous weeds during the reproductive stage of development affects the germination behavior of seeds by reducing the seed viability (Madafiglio *et al.* 2006). Herbicide application may have effects on the subsequent germination of seeds derived from herbicide treated weeds. The information on effect of post-emergence herbicides, *viz.* 2,4-D, carfentrazone-ethyl and metsulfuron plus sulfosulfuron various physiological, seed production potential and seed quality parameters of *M. denticulata* is lacking, when applied at different growth stages. Also, the potential of these herbicides in affecting germination of *M. denticulate* seeds derived from herbicide treated plants is also not known. Therefore, present study was undertaken with the objective to evaluate the effect of weed growth stage at the time of herbicide application on efficacy of different post-emergence herbicides and possible herbicide carryover effects onto future generation.

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MATERIAL AND METHODS

General information

Field experiments were conducted during *Rabi* 2016-17 and 2017-18 at Research farm of Punjab Agricultural University (PAU), Ludhiana, India. The experimental field had been under irrigated rice-wheat cropping system. The seedbed was prepared by one ploughing with disc harrow followed by two ploughings with cultivators and each ploughing was followed by planking. Wheat (cv. *PBW-677*) was sown during November 2016 and 2017 at a row spacing of 22.5 cm using 100 kg seed rate/ha. The seeds of *M. denticulata* were broadcasted uniformly in the field before sowing of wheat crop. Experiment was laid out in split plot design replicated thrice, with three growth stages of *M. denticulate, viz.* four, eight and twelve-leaf stages as main-plot treatments and seven weed control treatments as subplot, *viz.* 2,4-D sodium salt at 250 and 500 g/ha, carfentrazone-ethyl at 10 and 20 g/ha, pre-mix herbicide metsulfuron-methyl plus sulfosulfuron at 15 and 30 g/ha and water sprayed control. Fifteen plants of *M. denticulata* with leaf-stage as per treatment was maintained in each experimental plot (2.5 × 4.0 m). The herbicides were sprayed using a knap sack sprayer fitted with flat fan nozzle at 4, 8 and 12 leaf stages of *M. denticulata* which corresponded to 35, 50 and 60 days after sowing of wheat crop.

The data of chlorophyll fluorescence and chlorophyll content index was recorded from tagged plants at flowering using chlorophyll fluorometer (Model - OS-30p, Opti-Sciences, Inc.) and portable chlorophyll content meter (Model – CCM-200, Opti-Sciences, Inc.). For recording the observations, the middle portion of the leaf was dark-adapted with plastic clips before exposing to the light emitted by the fluorometer. The fluorescence readings were expressed as F_v/F_m (variable fluorescence/maximum fluorescence).

Density of *M. denticulata* was recorded from each plot at 20 DAS (days after spray) and was expressed as numbers of plants/m^2. For recording biomass, plants were cut; dried in sunlight and then placed in the paper bags for oven drying at 60 °C for 48 hours. Dry weight was taken till constant weight was achieved. The data was later expressed in g/m^2. Weed control efficiency (WCE) was calculated as:

$$WCE = \frac{(WDC - WDT)}{WDC} \times 100$$

Where, WDC = weed dry weight from control plot, WDT = weed dry weight from treated plot

At the maturity stage, five plants of *M. denticulata* were selected randomly from each plot for recording the number of fruits and seeds per plant.

Seeds collected from water sprayed control and herbicide treated plants of *M. denticulate* were tested for following seed quality parameters. Germination counts were daily made for 15 days after start of the experiment. The seeds showing visible protrusion of radicle were considered as germinated. Germination percentage was calculated as: Per cent Germination = [Number of seeds germinated /total number of seeds] × 100

Speed of germination (germination index) was calculated using the following formula given by the Association of Official Seed Analysts (1983)

$$GI = \frac{Number\ of\ germinated\ seeds}{Days\ of\ first\ count} + - - + \frac{Number\ of\ germinated\ seeds}{Days\ of\ final\ count}$$

Mean germination time (MGT) was calculated using the following equation of Ellis and Roberts (1981)

$$\text{MGT} = \frac{\Sigma\,(\text{Dn})}{\Sigma\,\text{n}}$$

Here *n* is the no. of seeds that had germinated on day D, and D is the no. of days counted from the beginning of germination.

The results of both the years were pooled before subjecting to ANOVA in randomized block design using statistical analysis software version 9.2 (SAS 2009). Means were separated at 0.05 using Fisher's Protected Least Significant Difference (LSD) test (Cochran and Cox 1966).

RESULTS AND DISCUSSION

Crop yield

The highest and lowest grain and straw yield of wheat were recorded when herbicides were sprayed at four and twelve-leaf stages of *M. denticulata,* respectively (**Table 1**). Herbicide sprays at twelve-leaf stage during both years caused > 7 and 4% reduction in grain and straw yield, respectively than sprays done at four-leaf stage. The highest grain and straw yield during both cropping seasons were recorded in plots treated with 20 g/ha of carfentrazone-ethyl which remained at par with other herbicide treatments, *viz.* carfentrazone-ethyl 10 g/ha, metsulfuron-methyl plus sulfosulfuron at 15 and 30 g/ha and 2,4-D at 500 g/ha but significantly superior to 2,4-D at 250 g/ha and unweeded control. Biological yield was similarly affected as that of grain and straw yield, with the highest biological yield being

Table 1. Effect of weed and crop growth stage at the time of herbicide spray and different herbicides on yield of *Triticum aestivum* L. (pooled data of 2016-17 and 2017-18)

Treatment	Biological yield (t/ha)	Grain yield (t/ha)	Straw yield (t/ha)
Weed growth stage at the time of herbicide spray			
4 leaf stage (35 days)	13.22	5.48	7.74
8 leaf stage (50 days)	12.55	5.13	7.42
12 leaf stage (60 days)	12.30	5.00	7.26
LSD (p=0.05)	0.24	0.11	0.13
Herbicide treatment			
2,4 D sodium salt 250 g/ha	12.48	5.11	7.37
2,4 D sodium salt 500 g/ha	12.71	5.22	7.49
Carfentrazone-ethyl 10 g/ha	12.81	5.27	7.53
Carfentrazone-ethyl 20 g/ha	12.91	5.31	7.60
Metsulfuron + sulfosulfuron 15 g/ha	12.72	5.22	7.50
Metsulfuron + sulfosulfuron 30 g/ha	12.77	5.25	7.52
Untreated control	12.41	5.07	7.34
LSD (p=0.05)	2.02	0.12	0.13
Interaction LSD (p=0.05)	NS	NS	NS

produced by 20 g/ha of carfentrazone during both years. The results of present study revealed that carfentrazone-ethyl and metsulfuron methyl plus sulfosulfuron at both 0.5X and 1X dose were equally effective in increasing yield and yield attributes of wheat over untreated control. However, 0.5X dose of 2,4-D was ineffective in improving productivity of wheat.

Chlorophyll fluorescence

The interaction effect of weed growth stage at the time of herbicide spray and different herbicides was found significant on chlorophyll fluorescence of *M. denticulata* (**Table 2**). *M. denticulata* plants treated with different herbicides at four-leaf stage recorded least F_v/F_m values. Whereas, chlorophyll fluorescence recorded highest values in plants treated with herbicides at twelve-leaf stage. Complete mortality of plants treated with 10 and 20 g carfentrazone at four-leaf stage was observed and hence no F_v/F_m values could be recorded in these plants.

Table 2. Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on chlorophyll fluorescence (F_v/F_m) of *Medicago denticulata* Willd. (pooled data of 2016-17 and 2017-18)

Herbicide treatment	Weed growth stage at the time of herbicide spray		
	4 leaf	8 leaf	12 leaf
2,4 D sodium salt 250 g/ha	0.700	0.749	0.757
2,4 D sodium salt 500 g/ha	0.698	0.749	0.755
Carfentrazone-ethyl 10 g/ha	0.00*	0.748	0.755
Carfentrazone-ethyl 20 g/ha	0.00*	0.747	0.756
Metsulfuron-methyl + sulfosulfuron 15 g/ha	0.356	0.746	0.754
Metsulfuron-methyl + sulfosulfuron 30 g/ha	0.347	0.746	0.754
Untreated control	0.703	0.749	0.758
Interaction LSD (p=0.05)		0.050	

* No F_v/F_m values recorded due to complete mortality of plants

Chlorophyll fluorescence (F_v/F_m) provides a measure of PSII photochemical efficiency and reflects the potential photochemical capacity of PSII. High values of F_v/F_m indicate high light transformation rate, providing more energy for CO_2 assimilation in dark reaction of photosynthesis. The herbicide application may block synthesis/cause degradation of photosynthesis related intermediate metabolites and affect fluorescence emission (Varshney *et al.* 2015). A lower value of F_v/F_m indicates that a proportion of PSII reaction centers are damaged, a phenomenon called photoinhibition, often observed in plants under stress conditions (Hess 2000, Hiraki *et al.* 2003). Reithmuller *et al.* (2003) reported that application of metsulfuron-methyl resulted in significant reduction in chlorophyll fluorescence of black nightshade (*Solanum nigrum*) and redshank (*Polygonum persicaria*).

Chlorophyll content index

Chlorophyll content index of *M. denticulata* was significantly influenced by both growth stage of *M. denticulate* by different time of herbicide spray and herbicides (**Table 3**). At flowering, *M. denticulata* plants sprayed at eight and twelve-leaf stages recorded higher values of chlorophyll content index which were statistically at par to each other. Whereas, complete mortality with carfentrazone application at four-leaf stage indicated higher sensitivity of younger growth stages of weed to this herbicide.

Table 3. Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on chlorophyll content index (CCI) of *Medicago denticulata* Willd. at flowering stage at PAU, Ludhiana (pooled data of 2016-17 and 2017-18)

Herbicide treatment	Weed growth stage at the time of herbicide spray		
	4 leaf	8 leaf	12 leaf
2,4 D sodium salt 250 g/ha	7.4	12.5	13.4
2,4 D sodium salt 500 g/ha	7.2	12.4	13.4
Carfentrazone-ethyl 10 g/ha	0.0*	12.5	13.2
Carfentrazone-ethyl 20 g/ha	0.0*	12.3	13.1
Metsulfuron-methyl + sulfosulfuron 15 g/ha	1.4	12.5	13.4
Metsulfuron-methyl + sulfosulfuron 30 g/ha	0.9	12.4	13.4
Untreated control	7.6	12.6	13.5
Interaction LSD (p=0.05)		1.40	

* No CCI values recorded due to complete mortality of plants

Chlorophylls are the essential photosynthetic pigments in plants and the amount of chlorophyll per unit leaf area indicates the overall condition of plants (Silla *et al.* 2010). There is a direct relation between chlorophyll content and light transformation in photosynthesis. The decrease in chlorophyll content due to herbicide application may be due to an increase of chlorophyll degradation or by reduction in chlorophyll synthesis (Santos 2004). It has also been reported that herbicide stress may induce reduction in the number of chloroplasts (Cakmak *et al.* 2009). Carfentrazone-ethyl is a diphenyl-ether herbicide, which is readily absorbed by foliage but has limited translocation. The herbicidal action of carfentrazone on susceptible plants involves inhibition of enzyme protoporphyrinogen oxidase which is involved in chlorophyll biosynthesis pathway. Initial symptoms appear as quickly as one day after treatment and plant mortality occurred within seven days of application (Obenland *et al.* 2019). In present study also, carfentrazone-ethyl at 10 and 20 g/ha resulted in complete killing of *M. denticulata* within 7 days when sprayed at four-leaf stage; whereas no phytotoxicity was observed when herbicide sprays were done at eight or twelve leaf stages of this weed.

Weed biomass, density and weed control efficiency

Delayed application of all the herbicides at eight leaf stage resulted in significant increase in number of surviving plants of *M. denticulata* with concomitant increase in weed biomass than herbicide sprays done at four-leaf stage during both years (**Table 4**). Carfentrazone-ethyl application at 10 and 20 g/ha to four-leaf stage of *M. denticulata* resulted in complete mortality with minimum biomass and > 95% weed control efficiency, whereas its delayed application at eight and twelve-leaf stages resulted in significant increase in number of *M. denticulata* plants with increased biomass leading to reduced efficiency. Similarly, 2,4-D and metsulfuron-methyl plus sulfosulfuron sprayed at both the doses were also more effective in reducing weed density and biomass of *M. denticulata* when applied at four-leaf stage than their application at eight and twelve-leaf stages.

Delayed application of all the herbicides at eight leaf stage resulted in significant increase in number of surviving plants of *M. denticulate* than herbicide sprays done at four-leaf stage during both years (**Table 3**). Carfentrazone-ethyl application at 10 and 20 g/ha to four-leaf stage of *M. denticulata* resulted in complete mortality with > 90% weed control efficiency, whereas its delayed application at eight and twelve-leaf stages resulted in significant increase in number of *M. denticulata* plants leading to reduced efficiency. Density of *M. denticulata* was statistically similar in response to 2,4-D application at either eight or twelve-leaf stage. Whereas, carfentrazone-ethyl and metsulfuron plus sulfosulfuron sprayed at twelve-leaf stage recorded significant increase in number of surviving plants of *M. denticulata* as compared to sprays done at eight-leaf stage.

Greater susceptibility of weeds at earlier growth stages as compared to later growth stages is because of rapid herbicide translocation *via* plasmodesmata during earlier stages (Kieloch and Domaradzki 2011). Size exclusion limit is a major factor which determines the size of molecules that can pass through plasmodesmata and therefore allows only restrictive macromolecular transport (Yadav *et al.* 2014). In older plants, size exclusion limit of plasmodesmata is reduced to > 50 times as compared to younger plants suggesting it to be one of the major reasons for reduced susceptibility of older plants to

Table 4. Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on *Medicago denticulata* Willd. density, weed control efficiency and biomass (pooled data of 2016-17 and 2017-18)

Treatment	Weed growth stage at the time of herbicide spray		
	Weed density (no. per plot)		
Herbicide treatment	4 leaf	8 leaf	12 leaf
2,4 D sodium salt 250 g/ha	13.33	14.83	14.83
2,4 D sodium salt 500 g/ha	7.66	14.16	14.00
Carfentrazone-ethyl 10 g/ha	0.00	12.16	14.50
Carfentrazone-ethyl 20 g/ha	0.00	11.83	14.00
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	8.50	13.33	14.50
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	5.33	13.00	14.16
Untreated control	15.00	15.00	15.00
Interaction LSD (p=0.05)		0.52	
Weed control efficiency (%)			
2,4 D sodium salt 250 g/ha	23.24	2.58	2.50
2,4 D sodium salt 500 g/ha	65.71	3.76	3.80
Carfentrazone-ethyl 10 g/ha	95.24	14.00	3.82
Carfentrazone-ethyl 20 g/ha	97.44	19.83	4.63
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	87.34	11.73	3.84
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	89.20	12.21	4.16
Untreated control	0	0	0
Interaction LSD (p=0.05)		2.41	
Weed biomass (g/plant)			
2,4 D sodium salt 250 g/ha	2.17	4.20	7.05
2,4 D sodium salt 500 g/ha	1.32	4.19	6.95
Carfentrazone-ethyl 10 g/ha	0.20	3.70	6.92
Carfentrazone-ethyl 20 g/ha	0.15	3.47	6.86
Metsulfuron-methyl + Sulfosulfuron 15 g/ha	0.47	3.83	6.87
Metsulfuron-methyl + Sulfosulfuron 30 g/ha	0.40	3.79	6.82
Untreated control	3.00	4.29	7.14
Interaction LSD (p=0.05)		0.37	

herbicides due to reduced translocation of herbicides (Concenco and Galon 2007). In present study, delayed application of carfentrazone at eight and twelve-leaf stages resulted in significant increase in number of surviving plants of *M. denticulata* as compared to carfentrazone sprayed at four-leaf stage. Results of present study are in agreement with Cauchy (2000) who reported that carfentrazone-ethyl was active at low dose rates (20 g/ha) and provided outstanding efficacy on a wider range of weeds with better results against young weeds, which were controlled within 1 to 2 weeks of herbicide application. Efficacy of auxinic herbicides has been reported to be reduced with delay in herbicide application (Eure *et al.* 2013). For example, Sellers *et al.* (2009) reported that control of dog fennel (*Eupatorium capillifolium*) was dramatically reduced when 2,4-D plus dicamba were applied to 154 cm tall plants as compared to 38 cm tall plants. In present study also 2,4-D was more effective when sprayed at four-leaf stage of *M. denticulata* than at eight and twelve-leaf stage.

Seed production potential

The seeds of *M. denticulata* are enclosed in coiled pods called burs (fruit) with seed number varying from 3-5 seeds per pod. The interaction effect of weed growth stage at the time of herbicide spray and different herbicides on seed production of *M. denticulata* was significant (**Table 5**). Maximum and minimum fruit and seed number per plant of *M. denticulata* was recorded with herbicide application at four and twelve-leaf stages, respectively. All the herbicides when applied at eight and twelve-leaf stages of *M. denticulata* caused significant decline in fruit and seed number than herbicide sprays done at four-leaf stage during both the years. *M. denticulata* plants sprayed with 30 g/ha of metsulfuron plus sulfosulfuron at eight and twelve-leaf stages recorded > 30 and 40 % decline in seed number/plant as compared to plants treated at four-leaf stage. Application of carfentrazone-ethyl at four-leaf stage of *M. denticulata* resulted in complete mortality of plants thereby completely inhibiting fruit and seed set. However, *M. denticulata* plants treated with carfentrazone-ethyl at eight and twelve-leaf stage were able to set seeds. Targeting weed seed production provides an effective tool for reducing the spread of herbicide-resistant weeds by preventing their establishment, spatial distribution and build-up of seed reservoirs in the soil seed bank (Bagavathiannan and Norsworthy 2012). Herbicide application at or near flowering or seed set has the advantage of decreasing weed seed production, eventually allowing the addition of lesser seeds in the soil seed bank in the next cropping seasons (Jha and Norsworthy 2012). Ganie *et al.* (2018) reported that single or sequential applications of 2,4-D or dicamba resulted in 96% inflorescence injury and reduction in seed production of giant ragweed (*Ambrosia trifida*) in the field as well as in greenhouse studies. The results indicated that 2,4-D or dicamba were effective options for reducing seed production of glyphosate-resistant *A. trifida* even if applied late in the season. Goroee and Saeedipour (2015) reported that metsulfuron plus sulfosulfuron at 30 g/ha was effective in suppressing seed formation in *Malva parviflora*.

Table 5. Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on *Medicago denticulate* Willd. fruit and seed number per plant (pooled data of 2016-17 and 2017-18)

Treatment	Weed growth stage at the time of herbicide spray Fruit number/plant		
	4 leaf	8 leaf	12 leaf
Herbicide treatment			
2,4 D sodium salt 250 g/ha	166	132	110
2,4 D sodium salt 500 g/ha	149	100	73
Carfentrazone-ethyl 10 g/ha	0*	149	129
Carfentrazone-ethyl 20 g/ha	0*	118	90
Metsulfuron-methyl + sulfosulfuron 15 g/ha	166	132	108
Metsulfuron-methyl + sulfosulfuron 30 g/ha	157	111	84
Untreated control	177	181	179
Interaction LSD (p=0.05)		5.41	
Seed number/plant			
2,4 D sodium salt 250 g/ha	664	528	440
2,4 D sodium salt 500 g/ha	596	400	292
Carfentrazone-ethyl 10 g/ha	0*	594	516
Carfentrazone-ethyl 20 g/ha	0*	472	358
Metsulfuron-methyl + sulfosulfuron 15 g/ha	662	528	432
Metsulfuron-methyl + sulfosulfuron 30 g/ha	626	444	334
Untreated control	706	724	716
Interaction LSD (p=0.05)		31.5	

* There was no fruit and seed set due to complete mortality of plants

Germination potential of *Medicago denticulata* seeds after herbicide exposure

Seeds collected from unsprayed plots (control) recorded higher germination as compared to seeds collected from plants treated with 2,4-D, carfentrazone-ethyl and metsulfuron methyl plus sulfosulfuron (**Table 6**). Application of herbicides at eight and twelve-leaf stage of *M. denticulata* produced seeds with decreased germination as compared to plants sprayed at four-leaf stage. However, there was no effect on time to start germination, speed of germination and mean germination time. In present study, *M. denticulata* seeds collected from unsprayed plots (control) recorded higher germination as compared to seeds collected from plants treated with 2,4-D, carfentrazone-ethyl and metsulfuron-methyl plus sulfosulfuron.

However, in contrast to our study, Tanveer *et al.* (2009) reported that *Chenopodium album* seeds collected from herbicide treated plants recorded higher germination as compared to seeds collected from unsprayed control plants. Qi *et al.* (2017) reported that *Amaranthus retroflexus* plants sprayed with atrazine or tribenuron-methyl both at vegetative and reproductive stages produced seeds with inhibited germination. Whereas in present study decreased germination of *M. denticulata* seeds was recorded only from plants sprayed at near reproductive (eight-leaf stage) or at reproductive stage (twelve-leaf stage). Wu *et al* (2016) reported that application of glyphosate and paraquat at late budding stage did not stop the growth of fleabane (*Conyza bonariensis*) plants which continued to develop, flower and set seeds. However, significant effects on seed viability and dormancy were recorded. Application of glyphosate alone or as a tank mix with pyraflufen-ethyl, glufosinate and flumioxazin has been reported to significantly reduce the germination percentage in red lentil (*Lens culinaris* L.) seeds compared to the untreated control (Subedi *et al.* 2017). It is important to note that present findings are more relevant only in the context of intended problem and objectives for making more balanced and wiser decisions in any given situation in general and delayed application of herbicides against the target weed(s), in particular.

Conclusion

Herbicide sprays done at four-leaf stage of *M. denticulata* provided effective weed control whereas delayed application resulted in poor control with no visual injury. Delayed application of all the herbicides at eight and twelve-leaf stage caused significant increase in weed density with concomitant decrease in weed control efficiency than herbicide sprays done at four-leaf stage. Poor weed control at advanced weed stages indicate importance of early herbicide application for effective control of *M. denticulata*. Seed production potential of *M. denticulata* was significantly decreased when herbicides were sprayed at advanced growth stages of weed. *M. denticulata* plants sprayed at eight and twelve-leaf stage survived the herbicide application; but great reduction in seed production was recorded as compared to herbicide sprays done at four-leaf stage. Seeds collected from plants sprayed at eight and twelve-leaf stages recorded decrease in germination as compared to seeds collected from plants sprayed at four-leaf stage. This indicates possibility of herbicide carry-over effect from parent plants.

Table 6. Interaction effect of weed growth stage at the time of herbicide spray and different herbicide treatments on germination potential of *Medicago denticulata* Willd. (pooled data of 2016-17 and 2017-18)

Treatment	Weed growth stage at the time of herbicide spray		
	Germination (%)		
	4 leaf	8 leaf	12 leaf
Herbicide treatment			
2,4-D sodium salt 250 g/ha	93.0	92.1	90.0
2,4-D sodium salt 500 g/ha	91.1	89.4	81.1
Carfentrazone-ethyl 10 g/ha	0*	88.3	86.1
Carfentrazone-ethyl 20 g/ha	0*	81.7	79.4
Metsulfuron-methyl + sulfosulfuron 15 g/ha	90.0	91.1	85.5
Metsulfuron-methyl + sulfosulfuron 30 g/ha	86.6	83.7	77.5
Untreated control	95.3	97.2	95.0
Interaction LSD (p=0.05)		4.23	
Speed of germination			
2,4-D sodium salt 250 g/ha	10.48	10.40	10.38
2,4-D sodium salt 500 g/ha	10.45	10.35	10.30
Carfentrazone-ethyl 10 g/ha	-	10.3	10.25
Carfentrazone-ethyl 20 g/ha	-	10.25	10.20
Metsulfuron-methyl + sulfosulfuron 15 g/ha	10.40	10.36	10.23
Metsulfuron-methyl + sulfosulfuron 30 g/ha	10.38	10.33	10.18
Untreated control	10.50	10.52	10.5
Interaction LSD (p=0.05)		0.25	
Mean germination time			
2,4-D sodium salt 250 g/ha	3.20	3.38	3.42
2,4-D sodium salt 500 g/ha	3.23	3.6	3.65
Carfentrazone-ethyl 10 g/ha	-	3.56	3.78
Carfentrazone-ethyl 20 g/ha	-	3.62	3.80
Metsulfuron-methyl + sulfosulfuron 15 g/ha	3.23	3.66	3.85
Metsulfuron-methyl + sulfosulfuron 30 g/ha	3.26	3.70	3.90
Untreated control	3.18	3.20	3.20
Interaction LSD (p=0.05)		0.04	

* There was no fruit and seed set due to complete mortality of plants

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