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Original Article: Herbicides for control of metsulfuron resistant toothed dock (*Rumex dentatus*)

Rajender Singh Chhokar[1]*, Ramesh Kumar Sharma[1], Ankur Chaudhary[2], Gyanendra Pratap Singh[1]

[1] *Resource Management Unit, ICAR- Indian Institute of Wheat and Barley Research, Karnal, Haryana, India*
[2] *CCS Haryana Agricultural University, Regional Research Station, Uchani (Karnal), India*

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***Corresponding Author:**
Rajender Singh Chhokar
(rs_chhokar@yahoo.co.in)

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ABSTRACT

Rumex dentatus L. (toothed dock), a major broadleaf weed, is a severe problem of irrigated wheat in particularly no-till conditions of the rice-wheat system in India. Metsulfuron is being used for its control. However, the sole reliance on metsulfuron has led to its resistance evolution in *R. dentatus*. Pot bioassay studies confirmed high level of metsulfuron resistance with GR_{50} values ranging 74 to 98 times to that of the most susceptible population. Metsulfuron resistant (MR) populations had indicated cross-resistance to other ALS inhibitor herbicides, triasulfuron, pyroxsulam, and florasulam. The ready-mix combination of halauxifen methyl + florasulam was also poor against MR populations. The most sensitive and resistant populations had GR_{50} values of 0.11 and 10.31, 1.0 and 269.5, <0.94 and >30, <1.13 and 3622.5, 0.16 and 10.14 g/ha for metsulfuron, triasulfuron, florasulam, pyroxsulam, and pre-mix of halauxifen + florasulam, respectively. Results showed a high resistance level in *R. dentatus* against ALS inhibitor herbicides. In addition, a large number of populations (119) were also screened across broad herbicide groups used in wheat and 68.9 % populations exhibited ≤ 50 % control with a recommended field rate of metsulfuron 4 g/ha, indicating the widespread resistance. However, MR- populations were sensitive to pendimethalin, isoproturon, 2,4-D, fluroxypyr, metribuzin, carfentrazone and flumioxazin. So, these herbicides in rotation and as mixture can be used to manage ALS inhibitor herbicide resistance. However, for long-term sustainable management of herbicide-resistant *R. dentatus*, alternative herbicides should be integrated with best agronomic practices to restrict its infestation in wheat.

Introduction

Toothed dock (*Rumex dentatus* L.) is a major winter season broadleaf weed of irrigated wheat in India. Its mature plants are generally 30-50 cm taller than wheat with an average plant height of 160±20 cm and produce abundant seeds (16000 fruits/seeds per plant) (Dhawan, 2005). It is highly competitive weed because of its vigorous growth and can reduce wheat grain yield by 70% with infestation of 30 plants/m^2 (Waheed *et al*., 2017). Its occurrence in wheat is mainly confined to rice-wheat system (Chhokar *et al*., 2007b; Singh *et al*., 1995) and further favored by adopting no-till (NT) in wheat (Chhokar *et al*., 2007a). The two favorable conditions for this weed, higher soil moisture and shallow seed burial (0-2 cm) are being provided by combination of NT-wheat and rice-wheat system.

The majority of *R. dentatus* emerges from seeds lying within shallow soil depth of 0-2 cm and fails to emerge with seeding depth of >4 cm (Dhawan, 2005; Singh and Punia, 2008). Although, the presence of perianth around the seed acts as a barrier to germination, but it also helps in concentrating the seeds near to surface during the puddle flooding conditions of rice due to lower seed density (Chhokar *et al*., 2007a). Moreover, its seed can tolerate the flooding conditions and a flooding duration of 80 days resulted in lowering the emergence of *R. dentatus* by 46% only (Singh and Punia, 2008). Thus, the abundant seed production, longer seed viability in soil (Lewis, 1973), ability to concentrate seeds near soil surface and withstand flooding conditions made it to fit well in rice-wheat system. Moreover, seeds are less prone to be destroyed by predators (Kumar *et al*., 2013) and as a result if not appropriately controlled, its abundance increases over the years.

It was not a major problem before the mid-nineties in India, when isoproturon and 2,4-D either alone or in combinations were mainly used for weed control in wheat. These herbicides kept under check the majority of broad-leaved weeds including *R. dentatus* (Chhokar *et al*., 2012). However, weed flora shifted in favor of this weed with shift in herbicide usage pattern after the evolution of isoproturon resistance in *Phalaris minor* Retz during the mid-nineties (Chhokar *et al*., 2012). The grass herbicides (fenoxaprop and clodinafop) and broad-spectrum herbicide sulfosulfuron recommended for control of single dominant weed isoproturon resistant *P. minor*, favored *R. dentatus* due to their ineffectiveness against this weed as well as removal of competition from other weeds. Simultaneously, the introduction of NT wheat in rice-wheat system of northwestern Indian plains further increased *R. dentatus* infestations because of favorable conditions in the system (Chhokar *et al*., 2007a). In NT system, it germinates before or along with the crop and can offer stiff competition as weeds germinating earlier or along with the crop are more competitive than late emerging.

For several decades, herbicides have been the preferred weed management tool. Among herbicides, one of the most important mode of action group of herbicides is ALS/AHAS inhibitors, which includes five classes of herbicides: sulfonylureas (SUs), imidazolinones (IMIs), triazolopyrimidinyl-thio-benzoates (PTBs), triazolo-pyrimidines (TPs), and sulfonylamino-carbonyl-triazolinones (SCTs) (Powles and Yu, 2010; Mehdizadeh, 2016). However, over-reliance on herbicides of limited modes of action of the risk-prone group such as ALS inhibitor herbicides has led to the quick evolution of herbicide resistance (Tranel and Wright, 2002; Beckie and Tardif, 2012; Delye *et al*., 2013). Among five ALS inhibitor groups, the first introduction is of SU herbicides group during 1980s and the first reported case of resistance from this group is of chlorsulfuron in a biotype of prickly lettuce (*Lactuca serriola* L.) with selection from chlorosulfuron + metsulfuron mixture in the USA in 1987 (Mallory-Smith *et al*., 1990). Currently, resistance to ALS/AHAS inhibitors has been documented in 168 weed species including 65 broad-leaved weeds from 54 countries (Heap, 2021).

Metsulfuron-methyl a highly active sulfonylurea herbicide is used globally at low rates ranging 2.0-5.0 g/ha (Chhokar *et al*., 2015). In India, metsulfuron at 4 g/ha was recommended during 1998 to control broad-leaved weeds including *R. dentatus* in wheat. The Indian farmers preferred metsulfuron over 2,4-D due to its better crop safety and compatibility with other grass herbicides (Pinthus and Natowitz, 1967; Balyan and Panwar, 1997; Chhokar *et al*., 2012). Therefore, metsulfuron was extensively used either in sequence with grass herbicides or as tank or ready-mixture with clodinafop and sulfosulfuron for diverse weed flora control in wheat. Since, *R. dentatus* was highly sensitive to metsulfuron, a reduced dose of 2.0 g/ha with sulfosulfuron 30 g/ha was also recommended (Chhokar *et al*., 2007b).

Metsulfuron remained effective for about 15 years in India and authors observed the first case of metsulfuron resistance evolution in a population of *R. dentatus* in 2013 (Heap, 2021) due to the continuous use of metsulfuron. Similarly, in Brazil, Argentina, Canada, and Australia, the strong selection pressure by continuous use of ALS inhibiting herbicides in wheat (*Triticum aestivum* L.) has documented the emergence of ALS resistant wild radish/radish (*Raphanus* sp.) biotypes (Yu *et al*., 2012; Pandolfo *et al*., 2013; Costa and Rizzardi, 2014; Cechin *et al*., 2016), causing crop yield losses and making the control costly and difficult. Now, in many fields, *R. dentatus* populations are escaping the control with metsulfuron even at higher doses (2X=8 g/ha) and repeated applications of the recommended rate leading to increased infestation in wheat fields. At few farmers' fields, its huge pressure is being observed leading to the problem in both manual and mechanical/combine harvesting of wheat crop along with significant yield losses. The similar maturity period of *R. dentatus* and wheat causes the contamination of the wheat grains with *R. dentatus* seeds. As most farmers use their own seed or neighbor farmers, which may further increase its infestation and the spread of resistance problem (Chhokar *et al*., 2012). The early detection and management of resistance is very crucial to avoid the yield losses. For herbicide resistance management alternative herbicides will always remain the central strategy. So, keeping these in view, the present study was undertaken to identify and quantify the herbicide resistance and cross-resistance patterns to various herbicides in *R. dentatus* along with its control by alternative herbicides.

Materials and Methods

A series of pot studies were conducted at Resource Management Block, ICAR- Indian Institute of Wheat and Barley Research, Karnal, Haryana, India (Latitude 29^{o} 43'N, Longitude 76^{o} 58'E at an elevation of 245 m above mean sea level).

Seed collection of Rumex dentatus populations

Mature seeds of seven *R. dentatus* populations were collected from wheat fields at maturity (month of April) during 2014 from Haryana state of India. Out of seven, five populations [Nagla, Kurukshetra (NAKUH); Saini Majra, Ambala (SMAMH); Ajrawar, Kurukshetra (AJKUH); Ujjha, Panipat (UJPAH) & Ajanthali, Karnal (AJKAH)] having inadequate or no control with metsulfuron methyl at the recommended (4 g/ha) or higher doses were collected from rice-wheat system. Seeds after collections were stored in paper bags at room temperature in the laboratory till November for bioassay studies. For comparison, populations (IIWBR research farm (DWR); Taraori, Karnal (TAKAH)) earlier confirmed sensitive to all herbicide assayed were used as susceptible (S) stock for resistance detection and quantification and these were from area having different herbicide applications and crop rotations history. The bioassay studies were conducted for three years and for seed collection, *R. dentatus* populations were raised separately in pots during 2014-15 and 2015-16. In addition, metsulfuron at 4 g/ha was sprayed on five resistant populations at around one-month old seedlings stage, whereas the susceptible population stock was maintained without any herbicide treatment. Moreover, after flowering stage, all the populations were kept in isolation, and also provided with a protected covering of a cloth to exclude any possibility of outcrossing till maturity. Once mature, the seeds were collected and kept separately for further studies in the subsequent season.

Quantification of herbicide resistance profile in R. dentatus

To quantify the herbicide resistance profile, pot studies were conducted for three consecutive *Rabi* seasons (2014-15, 2015-16, and 2016-17). Each year in November, *R. dentatus* populations were grown in pots of 15 cm diameter. The pots were filled with soil and well rotten farm yard manure (FYM) in 6:1 ratio by volume after passing through 2 mm sieve. The soil was from the field having no previous infestation of *R. dentatus* and was a sandy clay loam with pH of 8.1 and an organic carbon of 0.45%. The pots were watered to deplete the soil seed bank, if any, before sowing of *R. dentatus*.

R. dentatus seedlings were established by seeding about 60 healthy seeds (without perianth) at 0.5-1.5 cm depth. To remove the perianth, seeds were gently rubbed in a plastic tray. Three pots were used for each herbicide treatment (Table 1). Pots were labeled and arranged in a completely randomized design and watered as required. After three weeks of emergence, thinning was done to maintain fifteen plants per pot. Pre-emergence herbicide (Pendimethalin) was applied at 1-2 DAS (days after sowing) and post-emergence herbicides were sprayed 30-35 DAS (3 to 4 leaf stage of *R. dentatus*) with knapsack sprayer fitted with flat fan nozzles delivering 350 L/ha of water. For herbicide resistance profile studies, the herbicides and their graded doses used are given in Table-1. Cationic surfactant, Leader Mix (polyethylene amine) from Sumitomo India Ltd. was used at a concentration of 0.35% (v/v) with metsulfuron, triasulfuron, pyroxsulam, florasulam, and ready-mixture of halauxifen + florasulam treatments. Four weeks after the herbicide spray, the fresh weight of seedlings was recorded and, based on the biomass reduction, the 50 and 90% growth reduction (GR_{50} and GR_{90}) values were determined (Finney, 1971). GR_{50} could not be calculated for some resistant populations because fresh biomass did not decrease by 50% compared to control, even at the highest dose used. Therefore, GR_{50} and resistance index (RI) were mentioned in excess of the highest dose tested. Contrary, where the response at the lowest rate was higher than the 50 and 90% observed growth reduction, the GR_{50} and GR_{90} values were mentioned as less than the lowest tested dose except for metsulfuron where the calculated response was very close. The resistance index /Resistance Factor (RF) was calculated by dividing the GR_{50} values of different populations with GR_{50} value of the most S population. The values >2 were considered resistant (R).

Table 1. Herbicide rates for quantification of herbicide resistance in *R. dentatus*

Herbicide	Dose (g *a.i.* /ha)
Pendimethalin	0,125, 250, 500 and 1000
Metsulfuron	0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32 and 64,
Triasulfuron	0, 0.9375, 1.875, 3.75, 7.5, 15, 30, 60, 120, 240 and 480
Pyroxsulam	0, 1.125, 2.25, 4.5, 9, 18, 36, 72, 144, 288, 576, 1152 and 2304
Halauxifen 20.8% + Florasulam 20% WG	0, 0.1994, 0.399, 0.789, 1.59, 3.19, 6.38, 12.76, 25.52, 51.04 and 102.08
Florasulam	0, 0.94, 1.88, 3.75, 7.50, 15 and 30
Fluorxypyr	0,31.25, 62.5, 125, 250 and 500
Isoproturon	0, 125, 250, 500 and 1000
2,4-D E	0,62.5, 125, 250 and 500
Flumioxazin	0, 7.81, 15.62, 31.25, 62.5 and 125
Metribuzin	0, 12.5, 25, 50, 100 and 200
Carfentrazone	0, 2.5, 5, 10.0, 20 and 40

Screening of R. dentatus populations against major herbicide groups

Additionally, during two consecutive seasons of 2018-19 and 2019-20, a total of 119 populations collected were screened against six herbicides for knowing the extent of prevalence of metsulfuron resistance and any possibility of further extension of resistance to other mechanisms of action. The procedures for preparing pots and spraying herbicides were similar as mentioned in herbicide resistance profile studies section. However, the herbicide treatments evaluated in this study were metsulfuron at 4 g/ha, carfentrazone 20 g/ha, aclonifen + diflufenican 1000 g/ha, isoproturon 1000 g/ha, fluorxypyr 250 g/ha, 2,4-D-E 250, and 500 g/ha. These herbicides belonged to different chemical families namely sulfonylurea (metsulfuron-methyl), Aryl triazolinone (carfentrazone), urea (isoproturon), phenoxy-carboxylic acid (2,4-D), pyridine carboxylic acid (fluroxypyr), diphenylether (aclonifen), and pyridinecarboxamide (diflufenican). For comparison, untreated controls were also kept and compared to control of the respective population, the growth reductions and mortality under different herbicide treatments were visually assessed and populations were categorized into four groups comprising of 0-25, 26-50, 51-75, and 76-100% growth reductions. Finally, the percentage of the population falling under different % control group of the herbicide treatments was worked out.

Statistical analysis

The shoot fresh weight data of various *R. dentatus* populations were converted to percent biomass reduction in comparison to the untreated control and these data were pooled over the years due to similar response. The mean per cent growth reduction data were analyzed using non-linear regression procedure to estimate the mean herbicide dose causing 50 and 90% growth reduction (GR_{50} and GR_{90}) in a SAS 9.3 software (SAS Institute, Cary, NC).

Results and Discussion

Quantification of herbicide resistance profile

The resistance profile of seven *R. dentatus* populations (DWR, NAKUH, SMAMH, AJKUH, UJPAH, AJKAH, and TAKAH) was studied for three consecutive *rabi* seasons from 2014-15 to 2016-17 against twelve herbicides (Table 2) belonging to nine herbicide groups namely sulfonylurea (metsulfuron, triasulfuron), triazolopyrimidine sulfonanilide (pyroxsulam, florasulam), synthetic auxin/arylpicolinate (halauxifen methyl), pyridine carboxylic acid (fluorxypyr), phenoxy-carboxylic acid (2,4-D), triazolinone (carfentrazone), protoporphyrinogen oxidase/PPO (flumioxazin), phenylurea (isoproturon), and triazinones (metribuzin). Based on the mean responses across years of *R. dentatus* populations to different herbicides, the calculated GR_{50} and GR_{90} values are given in Table 2. The two biotypes (DWR, Taroari) were sensitive to all the evaluated herbicides. The 50% growth reduction (GR_{50}) values for metsulfuron were 0.12 and 0.11 g/ha for DWR and TAKAH, respectively, whereas 90% growth reduction (GR_{90}) value of DWR and TAKAH for metsulfuron was 0.34 and 0.37 g/ha, respectively. Both these sensitive populations recorded considerably lesser GR_{50} and GR_{90} values for metsulfuron than remaining five R populations having GR_{50} and GR_{90} values in the range of 7.75-10.30 g/ha and 49.84-104.61 g/ha, respectively. In comparison to the most susceptible response of a population (GR_{50} of TAKAH and GR_{90} of DWR) to metsulfuron, the resistant biotypes had GR_{50} and GR_{90} values of 73.8-98.2 and 146.6-307.7 times higher (Table 3). Thus, *R. dentatus* populations exhibited differential response to the application of metsulfuron. The most resistant (R) population (SMAMH) exhibited 308 folds greater GR_{90} value for metsulfuron than that of the most S population (DWR) and was equivalent to 26.1 times higher than the recommended field rate (X=4 g/ha) in wheat. Whereas, the 90% control (GR_{90}) of S populations was achieved even at 8.5-9.3% of the recommended rate. It means that highly metsulfuron sensitive (MS) *R. dentatus* has evolved a high level of resistance having RF as high as 308. Similarly, Costa and Rizzardi (2014) showed a very high-level RF (267- fold) of metsulfuron resistance in *Raphanus raphanistrum.*

Table 2. Herbicide resistance profile of *R. dentatus* populations based on response of three years (2014-15, 2015-16 and 2016-17)

Herbicides	GR_{50} /GR_{90}	Populations						
		DWR(S)	NAKUH	SMAMH	AJKUH	UJPAH	AJKAH	TAKAH(S)
Pendimethalin	GR_{50}	<125	<125	<125	<125	<125	<125	<125
	GR_{90}	135.1	143.3	142.6	123.9	130.2	142.6	157.4
Metsulfuron	GR_{50}	0.12	8.03	10.31	8.16	8.25	7.75	0.105
	GR_{90}	0.34	49.84	104.61	50.79	71.46	51.99	0.371
Triasulfuron	GR_{50}	1.06	141.1	269.5	170.36	175.73	158.27	1.0
	GR_{90}	2.31	>480	>480	>480	>480	>480	2.68
Pyroxsulam	GR_{50}	<1.125	1013.24	3622.46	2091.82	>2304	620.51	<1.125
	GR_{90}	1.42	>2304	>2304	>2304	>2304	>2304	1.376
Halauxifen + Florasulam	GR_{50}	0.18	8.45	10.14	7.811	6.51	5.81	0.155
	GR_{90}	0.52	64.78	74.48	56.92	71.11	36.21	0.602
Florasulam	GR_{50}	<0.94	>30	>30	>30	>30	>30	<0.94
Fluorxypyr	GR_{50}	<31.25	<31.25	<31.25	<31.25	<31.25	<31.25	<31.25
	GR_{90}	29.8	31.94	38.96	55.76	45.99	55.83	59.9
Carfentrazone	GR_{50}	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
	GR_{90}	5.71	5.2	5.4	5.7	5.0	5.9	6.9
Isoproturon	GR_{50}	<125	<125	<125	<125	<125	<125	<125
	GR_{90}	140.17	147.0	171.1	143.4	179.9	141.9	143.8
Metribuzin	GR_{50}	<25	<25	<25	<25	<25)	<25	<25
	GR_{90}	16.0	23.3	29.3	28.8	26.1	27.02	26.61
2,4-D-E	GR_{50}	69.9	83.18	80.24	90.99	71.82	75.06	82.7
	GR_{90}	226.1	213.0	252.8	238	232.0	282.2	245.9
Flumioxazin	GR_{50}	<7.81	<7.81	<7.81	<7.81	<7.81	<7.81	<7.81
	GR_{90}	<7.81	<7.81	<7.81	<7.81	<7.81	<7.81	<7.81

Table 3. Herbicide Resistance Index [RI= GR_{50}(R)/GR_{50}(S)] of *Rumex dentatus* populations for different ALS inhibitor herbicides

	Rumex dentatus Populations						
Herbicide	DWR(S)	NAKUH	SMAMH	AJKUH	UJPAH	AJKAH	TAKAH(S)
Metsulfuron	1.1	76.5	98.2	77.7	78.6	73.8	1.0
Triasulfuron	1.1	141.1	269.5	170.4	175.7	158.3	1.0
Pyroxsulam	1.0	>2048	>2048	>2048	>2048	>2048	1.0
Halauxifen + Florasulam	1.2	54.5	65.4	50.4	42.0	37.5	1.0
Florasulam	1.0	>31.9	>31.9	>31.9	>31.9	>31.9	1.0

The MS populations (DWR and Taraori) were also susceptible to triasulfuron, pyroxsulam, and florasulam (Table 2). However, metsulfuron R (MR) populations exhibited cross-resistance to triasulfuron, pyroxsulam, florasulam, and halauxifen-methyl + florasulam. The GR_{50} and GR_{90} requirement for triasulfuron of MR populations ranged from 141.1 to 269.5 g/ha and >480 g/ha, respectively. Five cross-R populations exhibited the GR_{50} values for triasulfuron >9.4-17.96 times (141.1-269.5 g/ha) of the field application rate (15 g/ha). Similarly, for pyroxsulam and halauxifen + florasulam, the GR_{50} values of MR populations ranged from 620.5to >2304 g/ha, and 5.81-10.14 g/ha (>551.6-3220 and 37.5-65.4 times of the most S biotype), respectively, while the respective GR_{90} requirements were >2304 g/ha and 36.21-74.48 g/ha. Thus, compared to S, MR populations needed >1674.4 folds higher pyroxsulam for the same level of 90% growth reduction responses. Furthermore, it is evident that the cross-resistance level was higher for pyroxsulam and florasulam, but lower for ready-mix combination of halauxifen + florasulam. Similarly, the high cross-resistance levels in *G. spurium* populations of ALS inhibitors (chlorsulfuron, tribenuron, florasulam, triasulfuron, thifensulfuron, and sulfometuron) have been shown by many weed scientists (Hall *et al*. 1998; Van Eerd, 2004; Beckie and Tardif, 2012). Earlier also, variable and higher SU resistance levels of radish were observed for chlorsulfuron having GR_{50} values greater than 640 g ha^{-1} (Han *et al*., 2012) and more than 43 g ha^{-1} of metsulfuron-methyl (Pandolfo *et al*., 2013).

In the present study, although the resistance in *R. dentatus* was observed with metsulfuron but the resistance level was higher with pyroxsulam, which is not yet used. This response might be due to the type of target site alteration selected by metsulfuron (Lamego *et al*., 2009). Contrarily, Kudsk *et al*. (1995) pointed the higher resistance to chlorosulfuron, the selecting agent compared to triazolopyrimidine herbicide (flumetsulam), whereas compared to metsulfuron higher RI was reported for other SU herbicides in Kochia (*Kochia scoparia* [L.] Schrad.) (Saari *et al*., 1990) and *Stellaria media* (Kudsk *et al*., 1995).

The triasulfuron, pyroxsulam, and halauxifen methyl + florasulam are not yet used by the wheat farmers in India because they are either under registration process or not commercially marketed (triasulfuron) for wheat crop. The expected cross-resistance pattern against these herbicides except ready-mixture of halauxifen-methyl + florasulam is because of similar mechanism of action (ALS inhibition). The cross-resistance in pre-mix halauxifen + florasulam is because halauxifen, a synthetic auxin, is poor against *R. dentatus* (Chhokar unpublished data). Mostly, if resistance evolves to a herbicide, then the other herbicides of that group usually become ineffective. Similarly, in the present study, the MR populations showed cross-resistance to triasulfuron, pyroxsulam, and florasulam.

Similar to the response of *R. dentatus* against pyroxsulam in the present studies, Sprague *et al*. (1997) reported the resistant *A. rudis* biotype >1920 folds more R to chlorimuron at the whole-plant level than the S biotype. The chlorimuron rate required to reach the GR_{50}, in the R biotype was not attained with rates as high as 1000 g/ha (100 times the field use rate). However, the GR_{50} for the S biotype was 0.52 g ha^{-1} much less than the labeled field rate of 10 g ha^{-1}.

The variable pattern of resistance and cross-resistance level within and between the various ALS inhibitor herbicides families is generally observed (Poston *et al*., 2000; Merotto *et al*., 2009; Pandolfo *et al*., 2013; Schaedler *et al*., 2013; Sada *et al*., 2013; Kuk *et al*., 2003) in different weed species/populations. This differential response occurs because ALS herbicide families bind to various domains in the enzyme. Thus, if resistance is due to alteration(s) in the binding site, then the affinity of ALS herbicides will be affected differently (Devine *et al*., 1991; Tranel and Wright, 2002). Resistant biotypes of *Lindernia* spp. showed very diverse RI values ranging from 60 to 14,100 (Uchino and Watanabe, 2002). Saari *et al*. (1992) studied four SU resistant weed species and found a higher resistance level to the selecting agent with only one species, *i.e*. perennial ryegrass. The greater RF with the SUs (thifensulfuron and chlorimuron) than with the imidazolinone (imazethapyr) at the whole plant level was observed in common waterhemp (Lovell *et al*., 1996), Russian thistle (Saari *et al*., 1992), and kochia (Saari *et al*., 1990) in spite of the selecting herbicide family was either SU (Saari *et al*., 1990, 1992) or imidazolinone (Lovell *et al*., 1996). In contrast, a smooth pigweed biotype selected with imazaquin demonstrated high resistance levels to imazaquin and imazethapyr, but little or negative cross-resistance to eight SU herbicides at the enzyme level (Manley *et al*., 1995). Likewise, chlorosulfuron R sugar beet was not cross-resistant to the metsulfuron, *imidazolinones, i.e. imazaquin and imazethapyr (Hart et al., 1992).*

Our studies indicate SU resistant R. dentatus as cross-resistant to TP. Earlier studies showed a general trend of cross-resistance between sulfonylurea (SU) and triazolopyrimidine (TP) herbicides, and between imidazolinone (IMI) and pyrimidinylthiobenzoate (PTB) herbicides (Devine and Eberlein, 1997). Whereas Mendes et al. (2019) found at least three distinct resistance patterns: resistance to imazethapyr/IMI, chlorimuron/SU (pattern R1), resistance to imazethapyr, chlorimuron, and diclosulam/TS (pattern R2) (the most frequent), and the exclusive resistance to imazethapyr (pattern R3) in Bidens spp. against ALS-inhibitors.

Previous studies showed the higher vulnerability of ALS inhibitor herbicides to resistance evolution and it can occur quickly with four to seven consecutive applications (Mallory-Smith et al., 1990; Primiani et al., 1990; Saari et al., 1994; Hashem et al., 2001; Beckie, 2006). However, even exposures to two applications of either SU herbicide (Sprague et al., 1997) or the imazethapyr (Hora and Peterson, 1995) have led to the appearance of resistance in populations of A. rudis. In our studies, about 10-12 years of metsulfuron usage resulted in resistance evolution. Tranel and Wright (2002) suggested that factors likely to enhance the selection of R-biotypes include "the repeated use of that herbicide over large areas, little or no use of the alternative modes of action herbicides, high efficacy of the herbicide on sensitive biotypes at the rate used, and soil residual activity of the herbicide. All these criteria match with the present case of MR- R. dentatus.

Besides, depending on the nature of herbicides, the resistance evolution also depends on the weed species. Amaranthus species are among the annual broadleaf weeds most prone to develop herbicide-resistant biotypes because of their high genetic variability, high production of rapidly germinating seed, and efficient pollen and seed distribution (Lovell et al., 1996). Similarly, the genus Rumex also shows high genetic variation, abundant seed production capacity and seeds can disperse through wind, water and contamination with crop seeds resulting in an increased selection pressure.

The other auxin herbicide 2,4-D-E had similar effectiveness against both the MR- and MS-*R. dentatus*. The GR_{50} values ranged from 69.9 to 91.0 g/ha. Although, auxinic herbicides have been in the market for almost 75 years, resistance to this mode of action has been reported in only 41 weed species to date (Heap, 2021). Also, various *R. dentatus* biotypes responded similarly for isoproturon, metribuzin, fluorxypyr, carfentrazone, and pendimethalin, and also exhibited the respective GR_{50} values for these herbicides across populations as <125, <25, <31.25, <2.5, and <125g/ha. Whereas GR_{90} values observed across populations were 140.2-179.9, 16.0-29.3, 29.8-59.9, 5.0-6.9, and 123.9-157.4 g/ha, for isoproturon, metribuzin, fluorxypyr, carfentrazone, and pendimethalin, respectively. Hamouzova *et al*. (2019) also observed no differential response of R- and S-populations, to isoproturon.

Our studies indicate *R. dentatus* populations as highly sensitive to pre-emergence application of pendimethalin and 90% control was achieved at about 16% of the field recommended rate of 1000 g/ha. The main reasons were shallow seeding and prevailing good moisture in pot studies. Gasper *et al*. (1994) also reported improved weed control with pendimethalin under better soil moisture conditions. Besides, pendimethalin, the excellent control of all *Rumex* populations was achieved after being treated with the recommended rates of carfentrazone, fluorxypyr, metribuzin, and 2,4-D. Similarly, Cechin *et al*. (2016) reported alternative post-emergence chemical control options for the ALS inhibitor resistant and cross-resistant radish as synthetic auxins (2,4-D amine), PSII inhibitor (bentazon), and PPO inhibitor (saflufenacil) in wheat crop. Hashem *et al*. (2001) also found that wild radish (*R. raphanistrum*) biotypes R to chlorsulfuron were susceptible to 2,4-D**.** Nevertheless, the rarity of evolved resistance in weeds to bromoxynil/ pendimethalin/ 2,4-D (Heap, 2021) highlights their future role in proactive and reactive herbicide resistant weed management in wheat.

Since, the rate of resistance development relates to the intensity of the selection for resistance (Martinez-Ghersa *et al*., 1997), the continuous usage of a herbicide or herbicides of the group having the same mechanism of action leads to faster herbicide resistance evolution associated with higher selection pressure (Burnet *et al*., 1991; Jasieniuk *et al*., 1996; Beckie, 2006; Powles and Yu, 2010). Similar situation might have happened in *R. dentatus* case, because the exclusive metsulfuron pressure was imposed in an unbroken rice-wheat sequence, which might have resulted in resistance development. The exclusive dependence on metsulfuron was because the farmers preferred it for its better efficacy against *R. dentatus* and safety to wheat crop compared to 2,4-D, as differential tolerance to 2,4-D depends on the wheat cultivars and the stage of application, as well. The repeated application of tribenuron in China has resulted in serious sulfonylurea resistance in main weed species *Descurainia sophia* (L.) Schur. (Cui *et al*., 2008). Likewise, NT and rice-wheat cropping sequence provide favorable conditions for heavy infestation of *R. dentatus* resulting in abundant seed production (Chhokar *et al*., 2007a). Heavy population pressure increases the chances of selection for R- populations as the number of herbicide R- mutants is proportional to population size (Jasieniuk *et al*., 1996). Moreover, metsulfuron is from high risk of herbicides (ALS inhibitors) and all these conditions favored resistance evolution. With the evolution of MR populations of *R. dentatus*, the interference in some wheat fields becoming similar to the herbicide resistant *P. minor* fields causing severe yield reductions to the extent of complete crop failure (Chhokar *et al*., 2012). Presently, the spread of metsulfuron resistance needs to be checked immediately; otherwise, it may become epidemic very fast and will threaten the food security by limiting the wheat production. One of the key tactics which can reduce the R- population is the early herbicide resistance detection and the use of alternative herbicides.

Based on the results of the present studies, the wheat yield reduction experienced in thearea having MR population can be resolved using pre-emergence pendimethalin and flumioxazin and post-emergence isoproturon, 2,4-D, carfentrazone, fluorxypyr, and metribuzin. In earlier studies (Chhokar *et al*., 2007b), 2,4-D, carfentrazone and isoproturon have effectively controlled S- *R. dentatus*.

Metribuzin was found equally effective in controlling both the herbicide R-and S-populations of *R. dentatus* (Table 2) and it is also effective against the other co-existing problem of multiple herbicide resistant *Phalaris minor* (resistant to ALS and ACCase inhibitors) (Chhokar *et al*., 2012). However, metribuzin adoption at farmers' field will depend on its selectivity on wheat cultivars, as the differential tolerance to metribuzin has been reported earlier (Runyan *et al*., 1982; Kleemann and Gill, 2007). Similarly, the effectiveness of triazine herbicides (atrazine) against Eastern black nightshade (*Solanum ptycanthum*) populations R to ALS inhibitors (imazethapyr, flumetsulam, cloransulam, nicosulfuron, prosulfuron, and rimsulfuron) was reported by Ashigh and Tardif (2006).

To control ALS R- populations of *R. dentatus*, pendimethalin can be used as pre-emergence in conventional-till system; where as in zero tillage (ZT) can be used in combination with non-selective herbicide like glyphosate/paraquat as a pre-seeding option. This pre-seeding herbicide combination will further improve the weed control as one partner will control the existing weed flush (glyphosate) and the other will provide the residual weed control (pendimethalin). Although, the dinitroaniline resistance has been reported in some weeds (Mudge *et al*., 1984; Heap and Knight, 1986; Vaughn *et al*., 1990; Morrison *et al*., 1991; Moss and Cussans, 1991), the extent of resistance is low mainly due to the paucity of a detoxification mechanism in weeds.

In another study, a total of 119 populations were tested against six herbicides of different group for knowing the extent of prevalence of metsulfuron resistance and any possibility of further extension of resistance to other mechanisms of action (Figure 1). Of the total population, 52.9, 16.0, 7.6, and 23.5% were classified as having 0-25, 26-50, 51-75, and 76-100% control with metsulfuron, respectively. A significant population (68.9%)

exhibited ≤50% control with metsulfuron 4 g/ha indicating large-scale infestation of MR- *R. dentatus* in wheat fields of north Indian Plains. The continuous monitoring of MR- *R. dentatus* including its distribution and spread is imperative for resistance management.

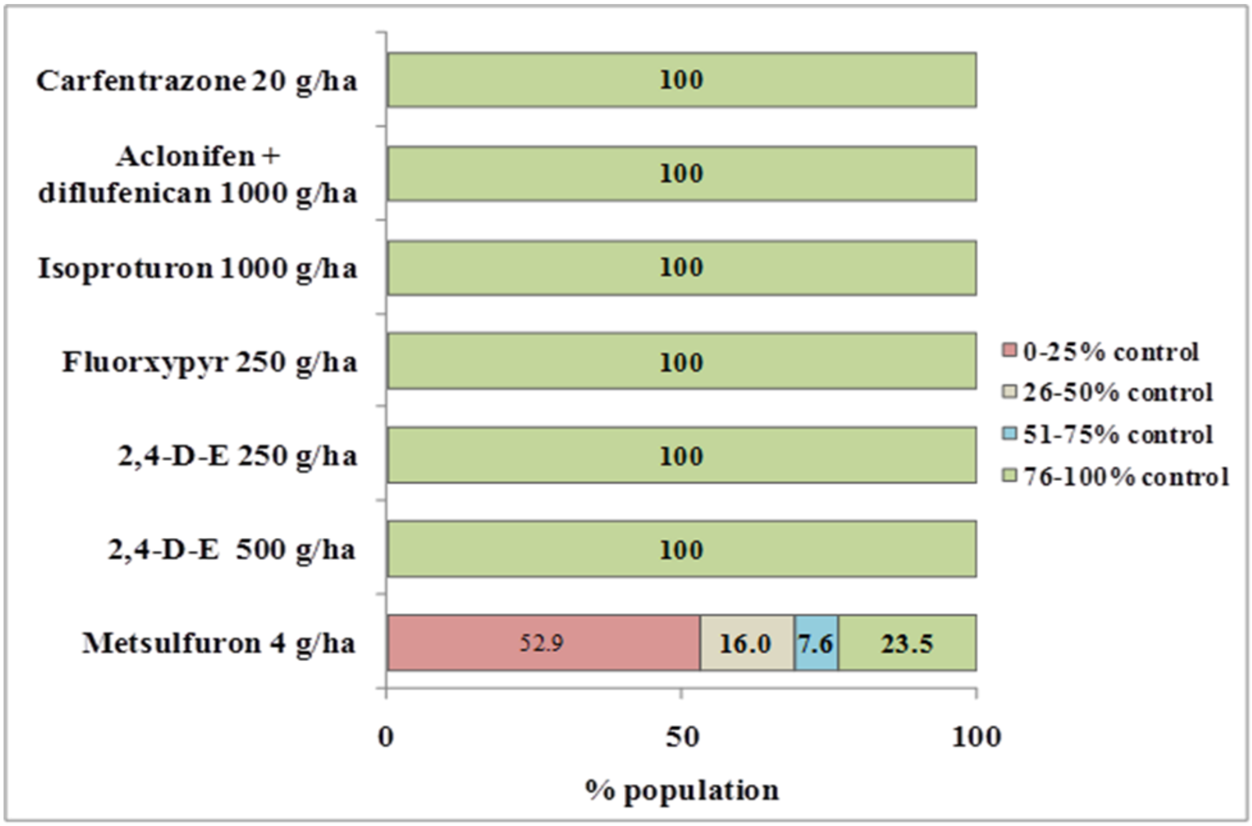

Figure 1. *Rumex dentatus* population percentage having different level of control with various herbicides during 2018-19 and 2019-20 (N= 119 populations)

While the alternative herbicides namely carfentrazone 20 g/ha, aclonifen + diflufenican 1000 g/ha, isoproturon 1000 g/ha, fluorxypyr 250 g/ha, 2,4-D-E 250, and 500 g/ha effectively controlled (76-100%) all the populations of *R. dentatus*. These herbicides belonged to different chemical families namely sulfonylurea (metsulfuron), triazolinone (carfentrazone), urea (isoproturon), phenoxy-carboxylic acid (2,4-D), pyridine carboxylic acid (fluroxypyr), diphenylether (aclonifen), and pyridinecarboxamide (diflufenican).

Herbicide group rotation and tank-mixing having different mechanism of actions are highly effective in preventing/delaying the resistance evolution and can be key component of an integrated weed management program (Jasieniuk *et al.*, 1996; Cavan *et al.*, 2000; Gressel, 2009; Beckie and Harker, 2017). A mixture of an ALS inhibitor with a photosystem-II inhibitor (Mallory-Smith and Retzinger, 2003) or auxinic herbicide or both is a much more effective tactic than rotation to delay resistance. Therefore, the alternative herbicides in mixture can be explored for managing and delaying the further extension of resistance. Alternative herbicide combinations such as isoproturon + 2,4-D, isoproturon + fluorxypyr, 2,4-D + carfentrazone, isoproturon + carfentrazone can be used for control of diverse broad-leaved weed flora including MR- *Rumex* in wheat. Brosnan *et al*. (2012) also reported that carfentrazone accelerates broad-leaved weed control with metsulfuron. However, for herbicide mixtures to be effective in delaying or preventing resistance, the component with the alternate mode of action should: (a) control the same spectrum of weeds; (b) have the same persistence; (c) affect a different target site; and (d) be degraded by different metabolic systems (Wrubel and Gressel, 1994).

Since no herbicide is invulnerable to selecting for resistant biotypes, excessive dependence on chemical weed control system is not a sustainable weed management strategy (Shaner, 2014). Thus, more focus has to be given to non-chemical weed control measures. Among non-chemical methods, the crop rotation is the most critical agronomic strategy to lower the selection pressure (Gressel and Segel, 1990) through implementation of different management options and restoration of diversity in weed flora. Crop rotation consisting of cover crops, competitive crops (barley/mustard) or short duration crops restricting weeds to form seeds due to difference in maturity period (pea and potato or the other vegetable crops between rice and wheat) or repeated cutting (green fodder crops like Egyptian clover, Lucerne, and Oat) will help in depleting the weed seed bank and thereby, the problem in the next season. Thus, crop rotation through its effect on weed seed germination and mortality affects the weed seed bank in soils.

Wheat varieties having early vigor with smothering and allelopathic effects on weeds also need integration. Adjusting the sowing time either early or late can also reduce *R. dentatus* impact. In Indian conditions, early wheat sowing (last week of October) reduces *R. dentatus* emergence along with crop due to higher temperature and less humidity leading to faster depletion of soil moisture in upper layers leading to reduced competition, whereas the late sowing deplete the soil seed bank by allowing germination followed by killing with herbicide or tillage operations.

Tillage also influences the weed flora abundance and it has been observed that NT favors the build-up of *R. dentatus*, but reduces the *P. minor* population. Similarly, conservation tillage and NT adoption increased the horseweed (*Conyza canadensis*) population and made it one of the most problematic weeds (Bhowmik and Bekeck, 1993; Brown and Whitwell, 1988). However, NT seeding also provides an opportunity to restrict the yield reductions due to control of herbicide resistant multiple weeds populations by pre-seeding application of tank-mix combination of non-selective herbicides (glyphosate/paraquat/glufosinate) with pendimethalin or metribuzin (Chhokar unpublished data). Moreover, if NT system with surface residue retention (conservation agriculture) is adopted then benefits are more in reducing the weed infestation (Chhokar *et al*., 2009). Kumar *et al*. (2013) also observed that residue retention drastically reduces the infestation of *R. dentatus* in wheat.

Among the various factors, the seed burial depth and soil moisture dramatically affect *R. dentatus* germination and emergence. For higher emergence, *R. dentatus* prefers higher soil moisture and shallow burial depth (Chhokar *et al*., 2007a; Singh and Punia, 2008). These findings need to be utilized for its management by creating unfavorable conditions through modifications in tillage crop establishment techniques involving zero-tillage, surface crop residue retention, and soil moisture management. Under rice-wheat system, after rice harvest, the major proportion of the *R. dentatus* seed bank remains near the soil surface. Allowing this seed bank to emerge and kill by either pre-planting herbicides combinations such as glyphosate/paraquat + pendimethalin in NT system or non-inverting shallow tillage in CT system can significantly lower its infestations. Moreover, the higher soil moisture requirement for seed germination of *R. dentatus* compared to wheat can also be utilized for its management. Chhokar *et al*. (1999) observed 64.7% germination of wheat even under osmotic potential of-10 bars, whereas *Rumex* germination is completely inhibited at osmotic stress higher than -2 bars (Dhawan, 2005). Thus, wheat could be seeded (5-6 cm depth) after slight depletion of moisture in the upper 3-4 cm soil layer of which *R. dentatus* emerges in maximum number, ultimately reducing the *R. dentatus* emergence. Similarly, burying *R. dentatus* seeds deeper than 4 cm by initial or primary tillage followed by subsequent shallow tillage operations so that seeds buried deeper do not come in the upper layer integrated with depleted soil moisture conditions in upper layer (3-4 cm) will reduce and delay the emergence leading to reduced competition to crop. To create the depleted soil moisture in upper soil layer, integration of early sown CT system and stale seed bed can be useful. In this scenario, the *R. dentatus* likely to emerge can also be controlled with application of pendimethalin just before first irrigation. Moreover, in north India, its population is increasing over the years along with increased number of infested fields. One of the reasons is sowing of contaminated wheat seeds with *R. dentatus* seeds. The combine harvesting is mainly responsible for its contamination with wheat grains due to their similar maturity. Likewise, seeds of new variety move very fast from farmer to farmer and pose risk of resistance spread through movement of contaminated seeds. The other factors which can also spread resistance are the use of un-rotten FYM. Measures should be taken to check the spread of R- populations to new areas by encouraging the use of certified seed and well rotten FYM.

With restricted discovery of new modes of action chemistries, growers have to face the issue of a reduced number of herbicide options in future. So, concerted efforts are required to prolong the effectiveness of the available/possible alternative herbicides by assisting them with other weed control practices. Moreover, developing herbicide resistant wheat to glyphosate and glufosinate will add to chemical control option and simplify the weed management by tackling multiple types of grass and broad-leaved weeds resistant to ALS, ACCase, and photosynthetic inhibitor herbicides in wheat (Harker *et al*., 2012; Chhokar *et al*., 2012). Thus, herbicide resistant/tolerant wheat will be an additional tool to the weed management toolbox to control herbicide resistant and tolerant weeds. It will also promote wheat-based NT and CA systems, leading to lower energy requirements. However, the long-term resistance management strategies should include the diversified IWM consisting of herbicide rotation, herbicide mixture, crop rotation, competitive variety, higher seed rate, closer spacing, tillage practices, sanitation practices (weed free crop seeds and manure), herbicide resistant varieties, and stale seedbed for sustaining wheat production.

A detailed study on *R. dentatus* biology is also required for further strengthening the herbicide resistance management by identifying the weak points in its life cycle and targeting them. Whenever and wherever possible, consideration should also be given to the use of mechanical weed control to remove weeds surviving the herbicide application before seed setting as well as use of weed seed harvesting/mechanical harvester for reducing the weed seed bank. The integration of all these approaches will lower the evolution and spread of herbicide resistance, ultimately improving wheat production and farm income.

Conclusions

The persistent and intensive metsulfuron use for *R. dentatus* control in wheat led to the evolution of ALS resistant populations in the northern Indian plains. Bioassay studies exhibited high level of metsulfuron resistance (RF of 308) in *R. dentatus* having survival up to 16-folds the recommended field dose (4g/ha). Furthermore, MR populations showed high but variable level of cross-resistance to multiple ALS-inhibiting herbicides thus eliminating several ALS groups (SU and PTB) options in resistant areas. However, old herbicides, 2,4-D and pendimethalin along with carfentrazone, fluorxypyr, and metribuzin are quite effective in controlling MR populations. These herbicides in combination and rotation can be used to reduce the selection pressure for resistance evolution and thereby wheat yield reductions. However, long term sustainable weed management should include the integration of chemical and non-chemical weed control methods.

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