



Herbicide resistance in *Rumex dentatus* against metsulfuron herbicide in Punjab and Haryana, India

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ABSTRACT

Over-reliance and continuous use of similar mode of herbicides lead to increase in selection pressure which resulted in evolution of herbicide resistance in weeds. Metsulfuron-methyl is used for the control of broad-leaf weeds in wheat since 1990s. There are reports of failure of control of *Rumex dentatus* with metsulfuron from farmers' fields in North-Western India. Pot studies were conducted at Punjab Agricultural University, Ludhiana during winter (*Rabi*) 2018-19 and 2019-20 to quantify the status and level of herbicide resistance in *R. dentatus* in Punjab and Haryana. *Rumex* populations were collected from farmers' fields in both years. Fifty six and 33 biotypes of *R. dentatus* from farmers' fields of Haryana and 6 and 19 biotypes from Punjab were collected in first and second year, respectively. Biotypes were screened using recommended dose of metsulfuron-methyl (5 g/ha) along with unsprayed in pot study. Results revealed that 38 out of 56 biotypes of *R. dentatus* collected from Haryana in first year were found resistant to metsulfuron whereas only one biotype showed resistance from Punjab. Further, 23 biotypes out of 33 biotypes collected in second year were found resistant from Haryana whereas 14 biotypes out of 19 were found resistant in Punjab. This indicated that *R. dentatus* has evolved resistance against metsulfuron-methyl in different regions of Punjab and Haryana.

INTRODUCTION

Wheat is the second most important food crop after rice in India with 99.78 mt production from 30.59 mha area and having productivity of 3.22 t/ha (Anonymous 2018). Punjab and Haryana states contribute 65% for wheat in central pool and recognized as food bowl for national food security. Weeds are the major biological constraints that halt potential productivity of wheat crop by competing for natural resources and impairing the quality of produce (Chhokar *et al.* 2012). The yield losses in wheat due to weed competition could be reached up to 60% (Rao *et al.* 2014) and also the complete failure under severe competition with resistant weed species (Malik and Singh 1995). The problem of broad-leaf weeds is emerging in areas where herbicides for controlling grass weeds are continuously being applied without supplementing with broad-leaf weed herbicides (Chhokar *et al.* 2015).

Rumex dentatus L. (toothed dock/ Jangli palak) is the dominant broad-leaf weed of wheat in rice-wheat cropping system. It is an annual plant of polygonaceae family having 30-70 cm long erect stem with branching habit from base. It is having long

emergence period, comparatively faster growth, and higher fecundity. Wheat is more prone to *R. dentatus* competition as compared to *Phalaris minor* because of its high biomass and fast growth than wheat (Singh *et al.* 2013, Singh 2012). The losses in the crop grain yield increased from 1.3% to 69.8% when density of *R. dentatus* increased from 5 to 30 plants/m² (Waheed *et al.* 2017). Metsulfuron was recommended at very low dose rate (2-4 g/ha) for weed control in wheat during 1988 that provided satisfactory control of *R. dentatus* and other broad-leaf weeds. Metsulfuron, a sulfonylurea herbicide is an ALS inhibitor, which inhibit the activity of acetolactate synthase enzyme which is required for the synthesis of branched chain amino acids namely isoleucine, valine and leucine. It caused the starvation of plants for these three amino acids (Whitcomb 1999). Therefore, disruption of protein synthesis and photosynthates transport resulted in death of susceptible biotype. But in resistant biotypes, plants develop mechanism to nullify the effect of herbicide. The mechanism of herbicide resistance may be due to either target-site (altered site of action or over-expression of gene) or non-target site (reduction in amount of herbicide that reaches the target site via

reduced uptake or translocation of herbicide, increased metabolism of herbicide, herbicide sequestration and/or decreased rate of herbicide activation) herbicide resistance (Powles and Yu 2010). Due to continuous use of Metsulfuron-methyl, evolution of herbicide resistance in *R. dentatus* against this herbicide in Panipat region of Haryana was reported (Chhokar *et al.* 2013). Moreover, this resistant biotype showed cross resistance to iodosulfuron, triasulfuron, florasulam, iodosulfuron-methyl-sodium, mesosulfuron-methyl, halauxifen + florasulam and pyroxsulam (Chhokar *et al.* 2018). In India, it was the first case of herbicide resistance in broad-leaf weeds and first case in *R. dentatus* globally. So, the present study was conducted to confirm the status and level of the resistance at farmers' fields in two adjoining states namely Punjab and Haryana, India.

MATERIALS AND METHODS

Survey was conducted at the end of wheat growing season in the months of March and April in 2018 and 2019 from the rice-wheat grown regions of Haryana and Punjab. The wheat fields were selected based on the reports of weed control failures from farmers' fields. The seeds of *R. dentatus* populations that survived after recommended dose of herbicide application or escaped even after the herbicide application was collected. The seeds were collected from the fields at least 20 to 30 feet inside the field edge to make sure that the putative resistant plants were treated with herbicide application. The seeds were threshed and placed in paper bags. Bags were marked with name of village and district along with their GPS location. During first year survey, total 56 biotypes of *R. dentatus* were collected from Haryana state comprising Kaithal (8), Kurukshetra (11), Karnal (14), Ambala (8), Panchkula (2), Panipat (3), Yamunanagar (5) and Jind (5). Only six biotypes were collected from Punjab comprising Jalandhar (1), Ludhiana (1), Barnala (2) and Sangrur (2) districts, where farmers reported poor weed control. In second year, the study was extended further and 52 biotypes were again collected from different regions of both the states. A total of 33 biotypes were collected from Haryana state comprising Panipat (2), Jind (4), Kaithal (4), Karnal (5), Kurukshetra (3), Ambala (4), Yamunanagar (2), Rohtak (2), Bhiwani (2), Hisar (1), Fatehabad (2) and Sirsa (2) districts. A total of 19 biotypes were collected from Punjab, covering Fatehgarh Sahib (2), Hoshiarpur (1), Ludhiana (2), Mohali (2), Patiala (3), Rupnagar (1), Saheed Bhagat Singh Nagar (3), Barnala (2), Abohar (1) and Sangrur (2) districts.

The study was conducted at Punjab Agricultural University, Ludhiana during winter (*Rabi*) 2018-19 and 2019-20. The experiment was carried out in completely randomized design (CRD) with six replications. For pot study, soil was taken from the Students' Research Farm area which was free from weeds and was not exposed to any herbicides from the last three years for filling the pots. The soil was first air dried followed by fine crushing and finally passed through a 2 mm sieve. Earthen pots with 25 cm diameter were filled with the mixture of sand, field soil and vermi-compost in the ratio of 2:3:1. The pots were properly watered before sowing and the weeds were allowed to germinate, which were uprooted regularly for a month to exhaust the weed seed bank of soil. All the *R. dentatus* biotypes were sown in the pots on 21 October 2018 and 24 October 2019 for winter 2018-19 and 2019-20, respectively. Twenty seeds of each biotype were sown per pot at a depth of 2-3 cm and pots were lightly watered just after sowing. Thinning was done after complete emergence and ten plants per pot were maintained. The recommended dose of metsulfuron-methyl (5 g/ha) was applied at 3-4 leaf stage of plants using 375 liters of spray volume. The spray was done with knapsack sprayer fitted with flat fan nozzle. Control pots were maintained as untreated or unsprayed. Dry shoot biomass was recorded from untreated control (DMC) and treated pots (DMT) at 30 days after treatment. Growth reduction was calculated as:

$$\text{Growth reduction (\%)} = \frac{\text{DMC} - \text{DMT}}{\text{DMC}} \times 100$$

Based on the growth reduction, locations of the resistant biotypes were plotted on the map of Haryana and Punjab jointly according to their geographic coordinates (latitude and longitude), which were noted down at the time of seed collection with the help of GPS land meter. The location points were plotted with the help of ArcGIS software.

RESULTS AND DISCUSSION

The results of pot study done in winter 2018-19 revealed that 34 out of 56 biotypes of *R. dentatus* collected from Haryana were found resistant to metsulfuron and the resistance pattern was observed almost similar in every district (**Figure 1**). Results revealed that biotype HR 16 which collected from Panipat district of Haryana recorded minimum growth reduction followed by biotype HR 26 which was also collected from Panipat district. This indicated that resistance was more dominant in the Panipat region. Similar findings were also reported by Chhokar *et al.* 2013, Singh (2016), where they reported the resistance in *R. dentatus* to metsulfuron-

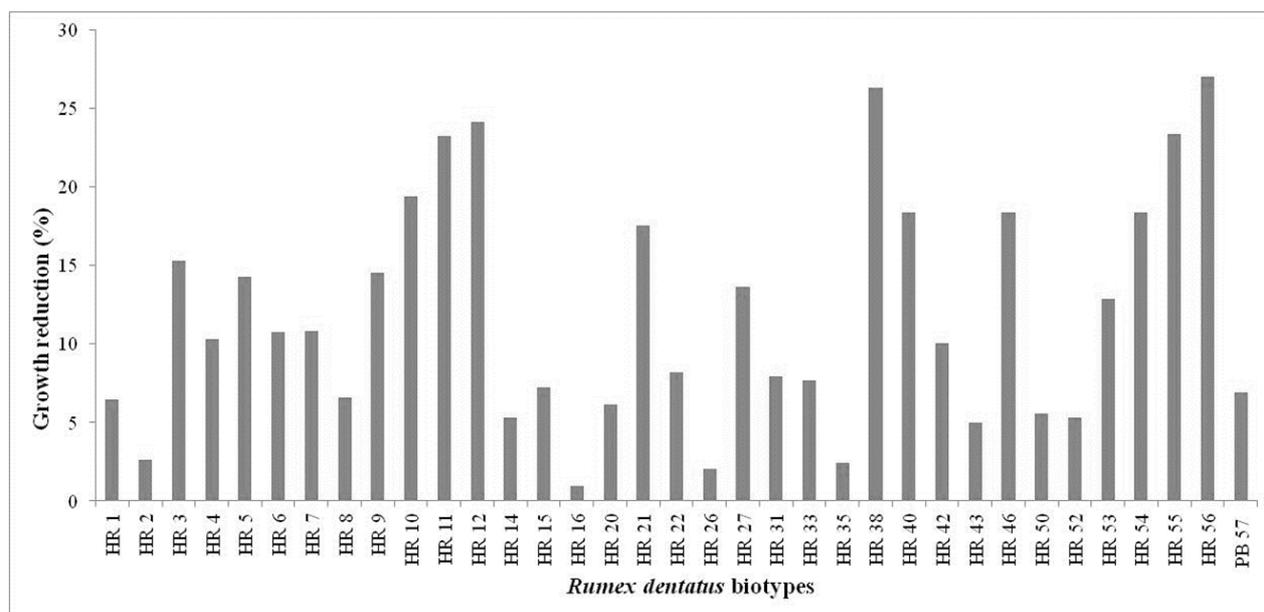


Figure 1. Growth reduction of resistant *R. dentatus* biotypes with Metsulfuron-methyl (5 g/ha) during 2018-19

methyl at farmer's field in Kaithal and Panipat districts of Haryana. In Punjab, only one biotype of *R. dentatus* collected from Barnala district showed resistance to metsulfuron. However, it is important to note that about 68 and 17% of the screened biotypes collected from Haryana and Punjab, respectively was defying action of metsulfuron-methyl and rest were susceptible. *R. dentatus* biotypes collected from Haryana namely HR 13 (Kakkarmajra, Ambala), HR 17 (Sharifgarh, Kurukshetra), HR 18 (Sirsala, Kurukshetra), HR 19 (Nissing, Karnal), HR 23 (Ragsana, Karnal), HR 24 (Habri, Kaithal), HR 25 (Hajwana, Kaithal), HR 28 (Mundwal, Kaithal), HR 30 (Rajankheda, Jind), HR 32 (Safidon, Jind), HR 34 (Kshindu, Panipat), HR 37 (Golimajra, Karnal), HR 39 (Manpura, Karnal), HR 44 (Potli, Yamunanagar), HR 48 (Badshami, Yamunanagar), HR 49 (Sonti, Kurukshetra), HR 51 (Jirbri, Kurukshetra) were found susceptible to the X dose (5 g/ha) of metsulfuron-methyl (Table 1). While, in Punjab biotypes collected from the Ludhiana, Sangrur and Jalandhar were found susceptible at X dose. During survey, conversation with farmers in Haryana revealed that putative resistant biotypes were mainly confined to fields where continuous rice-wheat system is being followed along with traditional yearly use of metsulfuron-methyl for controlling broad-leaved weeds in wheat. Moreover, farmers are using sub-lethal dose of metsulfuron (4 g/ha) in Haryana for spray. These factors could partially but enough to explain the evolution of herbicide resistance in *R. dentatus*. However, other factors such as poor spray techniques and delayed application of herbicide would have acted synchronically with above mentioned factors for this defying herbicidal action in Punjab.

The growth of resistant biotypes collected during second year was either unaffected or reduced slightly with spray of metsulfuron-methyl. Growth reduction was less (<30% growth reduction) in the resistant biotypes after the application of herbicide (Figure 2). Due to application of metsulfuron-methyl, less than 65% growth reduction was observed in nearly 70% of the screened biotypes of Haryana and 73% of screened biotypes of Punjab. More number of putative resistance biotypes was screened for the resistance from Punjab during the second year that subsequently resulted in more number of confirmed resistance biotypes as compared to first year. Further, the differential growth reduction of the biotypes were observed as some of the biotypes showed moderate resistance while others showed high resistance. This data showed the variability in resistance index of biotypes from different regions of Haryana and Punjab. The biotypes were classified into different groups of resistant (R) having <30%, moderately resistant (MR) having 30-65% growth reduction and susceptible (S) with >65% growth reduction (Table 1). A similar classification was adopted by Chhokar and Sharma (2008), Dhawan *et al.* (2010).

The map derived with the help of ArcGIS software clearly indicated that the resistant biotypes were mainly confined to Panipat, Kaithal, Kurukshetra and Karnal districts of Haryana and Fatehgarh Sahib, Patiala and Barnala districts of Punjab (Figure 3). Due to continuous application of metsulfuron herbicide for the control of broad-leaf weeds in wheat, *R. dentatus* showed resistance against this herbicide in many regions of Haryana as well as in Punjab state. Earlier, poor control of *R.*

dentatus was reported with metsulfuron-methyl and also with pre-mix application of sulfosulfuron + metsulfuron and mesosulfuron + iodosulfuron in Haryana (Chhokar *et al.* 2018). However, pre-mix application of metsulfuron + carfentrazone provided effective control of resistant *R. dentatus* than application of carfentrazone alone (Singh 2016). Yadav *et al.* (2017) also found that metsulfuron was not effective for controlling *R. dentatus* biotypes from Haryana (Panipat) even at 4 times of recommended dose (16 g/ha) and provided only 17% control at recommended dose (4 g/ha). However, 2,4-D (600 g/ha) and carfentrazone (20 g/ha) provided 98% and 87% control, respectively. But Chhokar *et al.* (2015) reported the comparatively poor control of *R. dentatus* in wheat with all the 2,4-D formulations. The order of effectiveness of three formulations was 2,4-D amine salt followed by 2,4-D ester and 2,4-D sodium salt. Application of carfentrazone, metsulfuron + carfentrazone and halauxifen-methyl + florasulam was found effective for controlling the resistant *R. dentatus*. Singh *et al.* (2017) also reported that herbicide resistance had evolved in *R. dentatus* to metsulfuron-methyl but found effective control with pre-mix application of

metsulfuron + carfentrazone. The resistance development in *R. dentatus* is likely to significantly impact the wheat productivity of this food bowl region of India as few herbicide options are available with farmers. Further, zero tillage area is likely to be increased in near future that subsequently makes more favorable ecological conditions for *R. dentatus*. As reports have shown that with the shift in tillage from conventional tillage to zero tillage, the propensity of *R. dentatus* infestation has increased significantly (Chhokar *et al.* 2007). Earlier studies indicated the sustainability issues in this region in the form of accelerated depletion of ground water, declining input factor productivity, imbalance application of fertilizers, adoption of monotonous cropping system (rice-wheat), crop residue burning along with accelerated evolution of herbicide resistance in wheat associated weeds (Humphreys *et al.* 2010, Chauhan *et al.* 2012, Chhokar *et al.* 2018, Chaudhary *et al.* 2019). There is a need to take the lesson from the accelerated development of cross and multiple resistance in *P. minor* within a short period of time from North-Western Indo-Gangetic plains to tackle the proliferation in *R. dentatus*.

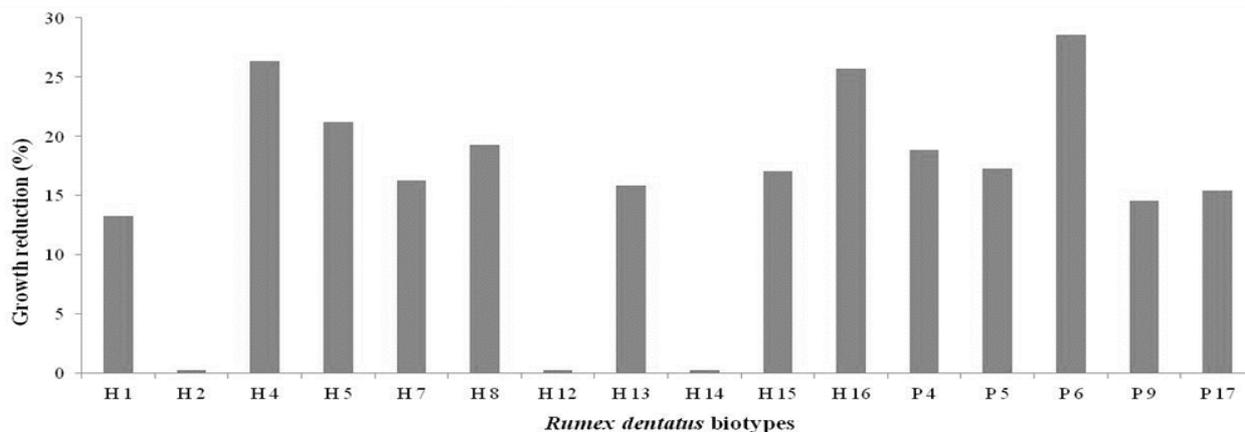


Figure 2. Growth reduction of resistant *R. dentatus* biotypes with metsulfuron-methyl (5 g/ha) during 2019-20

Table 1. Classification of biotypes according to their growth reduction under recommended dose of metsulfuron-methyl (5 g/ha)

Category	Haryana biotypes	Punjab biotypes
Winter (<i>Rabi</i>) 2018-19		
Resistant (R)	HR 1, HR 2, HR 3, HR 4, HR 5, HR 6, HR 7, HR 8, HR 9, HR 10, HR 11, HR 12, HR 14, HR 15, HR 16, HR 20, HR 21, HR 22, HR 26, HR 27, HR 31, HR 33, HR 35, HR 38, HR 40, HR 42, HR 43, HR 46, HR 50, HR 52, HR 53, HR 54, HR 55, HR 56,	PB 57
Moderately resistant (MR)	HR 29, HR 36, HR 41, HR 45, HR 47	-
Susceptible (S)	HR 13, HR 17, HR 18, HR 19, HR 23, HR 24, HR 25, HR 28, HR 30, HR 32, HR 34, HR 37, HR 39, HR 44, HR 48, HR 49, HR 51	PB 58, PB 59, PB 60, PB 61, PB 62
Winter (<i>Rabi</i>) 2019-20		
Resistant (R)	H 1, H 2, H 4, H 5, H 7, H 8, H 12, H 13, H 14, H 15, H 16	P 4, P 5, P 6, P 9, P 17
Moderately resistant (MR)	H 3, H 6, H 10, H 11, H 17, H 18, H 19, H 20, H 21, H 22, H 23, H 24	P 1, P 2, P 3, P 7, P 8, P 11, P 12, P 13, P 15
Susceptible (S)	H 9, H 25, H 26, H 27, H 28, H 29, H 30, H 31, H 32, H 33	P 10, P 14, P 16, P 18, P 19

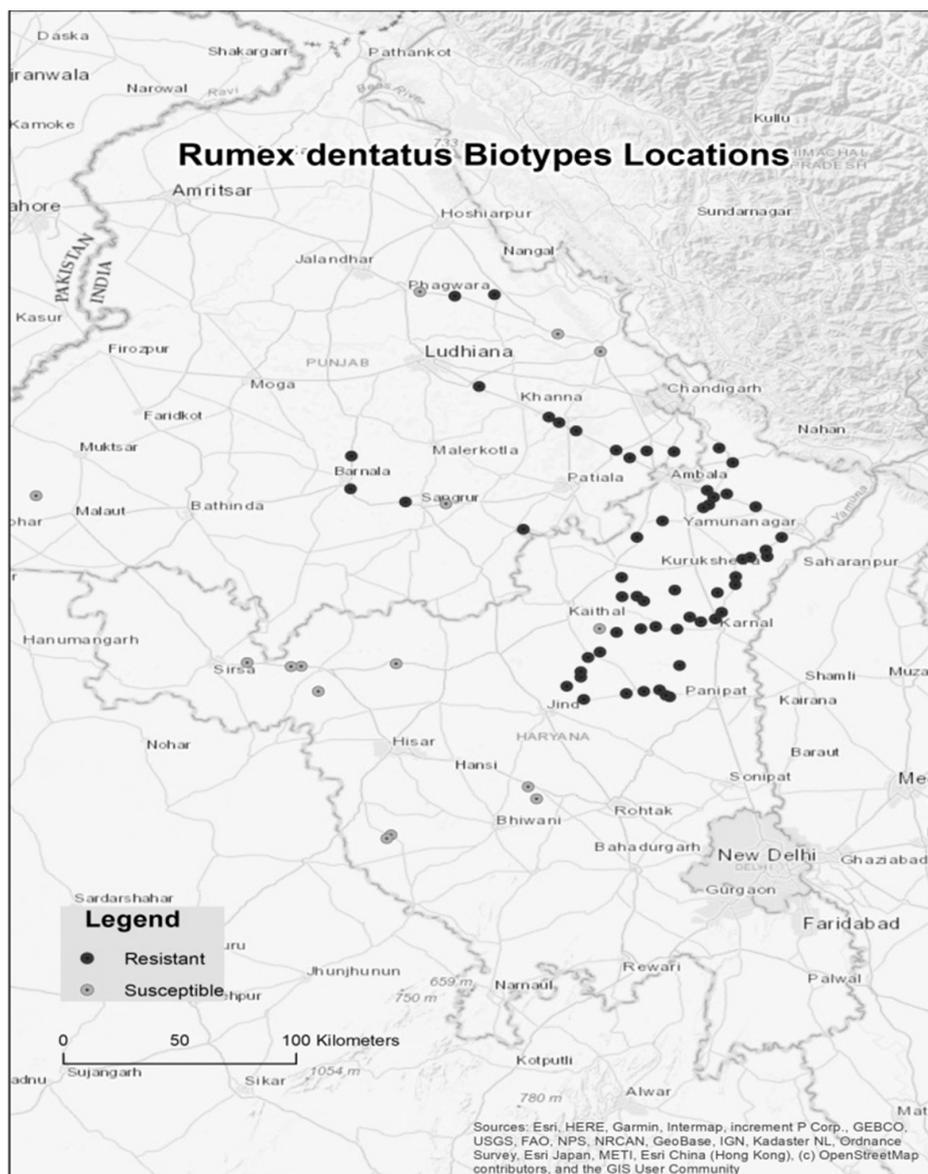


Figure 3. Map showing spread of resistance in *R. dentatus* biotypes collected from Haryana and Punjab

Based on two year studies, this is the first report of confirmation of resistance in *R. dentatus* not only in regions of Panipat but also in other areas such as Kaithal, Kurukshetra and Karnal districts of Haryana implying its wider infestation in the state which was lacking in previous studies. Further, this study also, testifies the infestation of metsulfuron-methyl resistant *R. dentatus* biotypes in Punjab for the first time. The use of metsulfuron-methyl is not economical for the control of *R. dentatus*, where resistance has been confirmed and likely to exacerbate the problem of resistance further. To control the resistant weeds, integrated weed management programs with herbicide of alternate mode of action should be recommended. This resistance is mainly confined in the rice-wheat

cropping system with continuous use of same herbicide for broad-laved weed control in wheat. The herbicide resistance in *R. dentatus* after *P. minor* in India is an alarming signal for sustainable wheat production. There is a need to characterize this resistance to decipher the mechanism involved, which helps in devising the new strategies for its management. The concerted efforts are required to understand the biology and ecology of *R. dentatus*, for developing suitable diversified approach to prevent the infestation and its seed production along with large scale adoption of preventing methods such as weed-free fields, use of certified weed free seeds, routine scouting, clean farm equipments free from weed seeds, restricted field-to-field and within-field

movement of weed seeds and harvesting of weed seeds should be followed to halt the infestation of *R. dentatus* in wheat. Further, field demonstrations should be conducted for creating awareness among farmers to improve the herbicides efficacy focusing especially about application of recommended dose of herbicides, with right spray nozzle, at right time with rotation of herbicides on yearly basis.

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