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Research Article

Herbicide resistant barnyardgrass in Iran and Turkey

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The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Highlights

- No resistance to ACCase inhibitors was seen in barnyardgrass biotypes collected from Iran
- One barnyardgrass biotype collected from Iran showed cross-resistance to penoxsulam and bispyribac-sodium
- Several barnyardgrass biotypes from turkey showed resistance to both ACCase and ALS inhibitors

Abstract

Background: Inconsistent control of Barnyardgrass has been reported repeatedly by farmers in major rice growing areas of Iran and Turkey.
Objective: Thus, a greenhouse study was conducted to investigate the resistance of Barnyardgrass to acetyl CoA carboxylase (cyhalofop-butyl) and acetolactate synthase (penoxsulam, bispyribac-sodium) inhibiting herbicides.
Methods: The seeds were sown in pots in a greenhouse and after screening, were sprayed with various rates of cyhalofop-butyl, penoxsulam and bispyribac-sodium herbicides at 3-4 leaf stage. Four weeks later, the above ground biomass was cut, dried in an oven and weighted. The results were then analyzed in the R software (drc package) using a four or three parameter log-logistic function. All experiments were repeated twice.
Results: While 30% of biotypes collected from Turkey were not controlled by ACCase inhibitors at twice the recommended rates, no resistance was observed in Iran's samples. Only one biotype exhibited cross-resistant to penoxsulam and bispyribac-sodium in Iran, which was due to consecutive application of herbicides with modes of action similar to these herbicides. In contrast, several Turkish biotypes showed cross-resistance as well as multiple resistance to ACCase and ALS inhibitors.
Conclusions: The rapid expansion of herbicide resistance in both countries indicate the necessity of adopting integrated weed management practices to hinder the further evolution of resistance in future.

1 Introduction

Rice has become the main crop produced in the eastern part of the world due to the key role it plays in dietary pattern of these regions. In 2013, around 493000 ha of rice were grown in northern parts of Iran, approximately 85% of Iranian rice production. In Turkey, 30000 ha of rice were grown in Samsun, Sinop and Corum, which was about 25% of total rice production in Turkey (FAO, 2018). Rice cultivation is quite different in these two countries. For example, in Iran transplanting and in Turkey direct seeding methods are widely practiced.

The genus of *Echinochloa* has 50 species (including subspecies and varieties) which are the most important weeds occurring in rice fields around the world (Andres et al., 2007). The broad ecological adaptation such as the ability to mimic rice, rapid germination, growth and abundant seed production make this species a successful weed in rice production system (Kaya Altop et al., 2014).

Echinochloa crus-galli possesses the C4 photosynthetic pathway, spreads and reproduces through seeds and is one of the world's most serious grass weeds (Rao et al., 2007). This weed can tolerate flooding or poor drainage, but is unable to bear serious drought (Bajwa et al., 2015). Yield losses caused by weeds may range from 15 to 42% depending on weed densities, weed species, seeding method and rice cultivar (Mennan et al., 2012). Bajwa et al. (2015) reported that *E. crus-galli* may reduce rice yield between 21 to 79%, depending on the management and cropping system.

Weed management is a major issue for rice producers. Application of herbicide is the best option to control *E. crus-galli* in Turkey and Iran. Currently the usage of this controlling system has decreased in Turkey and some other countries due to the elevated extent of herbicide resistance (Mennan et al., 2011). Rice-specific herbicides such as propanil were introduced in last 26 years. Since then, the number of registered herbicides to control weeds in rice has increased in Turkey and Iran. Some of these herbicides are single chemicals, but during last 5 or 10 years products with combination of two to three chemicals became popular. Recently, also, 'one-shot' (achieving desired control with a single usage) single herbicides including sulfonylurea compounds such as Inhibition of acetyl CoA carboxylase (ACCase), acetolactate synthase ALS (acetohydroxyacid synthase AHAS) and few others have been the most common. According to Heap (2020), *E. crus-galli* is reported to be resistant to many herbicides including cyhalofop-butyl (ACCase inhibitors, A/1), bispyribac-sodium and penoxsulam (ALS inhibitors, B/2), in various parts of the world, including Brazil, China, and Egypt, among others.

Due to consecutive application of herbicides common in rice cultivation, *E. crus-galli* has evolved resistance in northern provinces of Iran (Haghnama and Mennan, 2017). Thus, this study was conducted to examine *E. crus-galli* biotypes in terms of resistance to bispyribac-sodium, cyhalofop-butyl and penoxsulam, existence of multiple or cross resistance, assessment of resistance to herbicides commonly associated with rice production in Iran.

2 MATERIALS AND METHODS

2.1 Seed source

Mature seeds of *E. crus-galli* biotypes were collected from different rice growing regions of Iran and Turkey. In 2013, 56 different samples were collected from three provinces of Turkey and from the fields with a long history of herbicide use where weed control problems had been reported. Seeds were collected from field after full maturity. In Iran, seeds of 128 *E. crus-galli* populations were gathered from three provinces. All seeds were cleaned and stored in a laboratory to be used in the experiments. Susceptible biotypes were gathered from areas of each region with no history of being sprayed with the studied herbicides. It must be noted that the mechanisms responsible for the resistance of these biotypes had been determined previously (Haghnama, 2016).

2.2 Screening assay

In order to rule out the populations falsely reported as resistant, a single-dose experiment has been carried out according to Moss (Moss et al., 1999) to estimate possible resistance within 184 biotypes. If the herbicides effectiveness at recommended dose was less than 80%, these samples were involved in the subsequent dose-response experiments.

2.3 Dose-response experiments

Based on the single-dose-assay results, the putative tolerant as well as susceptible biotypes were seeded into cell trays containing commercial potting mix. Plants were grown at average daily temperatures ranging from 24 to 30 ºC and at a 16 h day length photoperiod and 80% relative humidity. After germination, seedlings were transferred into 0.3 L square plastic pots filled with rice paddy field soil. Each pot was fertilized with ammonium sulphate (21% N, 24% S) at rates of 350 kg ha^{-1} in two split doses before and after transplanting and first tillering, respectively. When seedling established, each pot contained 4 equidistantly spaced uniform plants and herbicides were applied at the 3-4 true leaf stages.

Penoxsulam (Cherokee™ OD 25.2 g a.i. kg^{-1}) was applied at rates of 0, 5.04, 10.08, 20.16, 40.32, 80.64, 161.28 and 322.56 g ha^{-1} *E. crus-galli* biotypes in Iran and Turkey after first screening tests. Bispyribac-sodium rates (Nominee™ 420 g a.i. kg^{-1}) used on the biotypes in were 0, 5.25, 10.50, 21, 42, 84, 168 and 336 g ha^{-1}. Cyhalofop-butyl (Clincher™ 200 g a.i. kg^{-1}) was applied at 0, 37.5, 75, 150, 300, 600, 1,200 and 2,400 g ha^{-1} to *E. crus-galli* biotypes from Turkey.

These rates correspond to 0, 0.25 0.5, 1, 2, 4, 8, and 16 times of the recommended field rate of the products. Herbicide treatments were applied with a single Teejet 8002EVS flat fan nozzle placed 30 cm above the target on a continuous link belt sprayer calibrated to deliver 300 L ha^{-1} at a pressure of 200 kPa. Dose response experiments for each herbicide were conducted based on completely randomized design with four replicates. Four weeks (28 days) after treatment (DAT), the aboveground biomass was harvested and dry weight were measured. All experiments were repeated twice.

2.4 Data analysis

Since no interaction was observed between the experimental runs and the herbicide treatments, the results of the two experimental runs were pooled.

Typically the data are best described with a four-parameter log-logistic curve (Ritz, 2010).

$$y = C + \frac{D-C}{1+\exp(b(\log(x)-\log(ED50)))}$$

in which y is biomass, C and D are lower and upper value of y, respectively, b is slope of the curve at ED_{50} point, which is the dose required to halve the biomass relative to D.

Since C parameter was not significant, the equation was altered to:

$$y = \frac{D}{1+\exp(b(\log(x)-\log(ED50)))}$$

Analysis of the dose-response curves was performed using R (version 2.15.2) with the add-on package drc (version 2.03.0). The multiple curves have been fitted simultaneously, as well as, each set quality of dose-response models has been differentiated with an analysis of variance through a lack-of-fit F-test (Seefeldt et al., 1995). The ED_{50} response level have been calculated from the model and graphical analysis of residuals has been used to estimate regression fits (Ritz, 2010).

Resistance index (RI) and Resistance-Susceptible (R/S) ratio were scored based on following formula (Ritz, 2010).

$$RI = \frac{\text{ED50 resistant biotype}}{\text{ED50 resistant biotype}}$$

Resistant biotypes of each province were evaluated with the susceptible biotypes collected from that same region.

3 Results and Discussion

The biotypes which maintained 80% dry weight compared to control under recommended dose of herbicide were chosen as resistant ones and underwent dose-response assay. No cyhalofop-butyl resistance was observed among biotypes collected from Iran, and only one biotype showed resistance to penoxsulam. Bispyribac-sodium resistance was more common though. Nevertheless, resistance was more severe in Turkey, and several biotypes proved to have developed resistance to studied herbicides (Tables 1 and 2).

Results of dose-response assay (Figures 1, 2 and 3) showed that the sole biotype resistant to penoxsulam in Iran (GOL-59) had a resistance factor of 7.07. Herbicide rates leading to 50% reduction in plant dry weight were 23.63- 85.41 g a.i. ha^{-1}. Thus, resistance factor of bispyribac-sodium resistant biotypes collected from Iran ranged from 2.21 to 8.00. Resistance factor and ED_{50} of Turkey biotypes which had evolved resistance to penoxsulam and bispyribac-sodium were respectively 37.31-60.76 g a.i. ha^{-1}, 2.30-3.74 and 38.86-70.31 g a.i. ha^{-1}, 2.05-3.70 (Table 1). Biotypes resistant to cyhalofop-butyl herbicide collected from Turkey showed ED_{50}s of 193.2-471.4 g a.i. ha^{-1}. Since the variation among susceptible biotypes collected from each province were relatively more than the ones associated with penoxsulam and bispyribac-sodium, the resistance factors were in some cases not in co-ordinance with ED_{50} values. Nonetheless, resistance factors ranged from 2.20 to 5.37 (Table 2).

According to Table 3, 4.1%, 8.2% and 34.9% of the biotypes collected from Turkey were resistant to penoxsulam, cyhalofop-butyl and bispyribac-sodium, respectively, and 54.14% showed cross resistance to bispyribac-sodium×penoxsulam. 4.4% of the biotypes were resistant to cyhalofop-butyl×penoxsulam, 27.7% showed resistance to cyhalofop-butyl×bispyribac-sodium, and bispyribac- sodium×penoxsulam× cyhalofop-butyl resistance was observed in 27.7% of the biotypes. 42.11% of biotypes were not characterized with multiple resistance.

Mennan et al., reported in 2012, 14% of the biotypes collected from Samsun and Corum were resistant and there were no reports of resistance in Sinop province. Our results indicated that resistant was confirmed at different frequencies for three used herbicides in the biotypes collected from three provinces of Turkey. However, all Iranian biotypes were susceptible to these herbicides at recommended dose because

Table 1 - Estimate of ED50 (herbicide rate required to cause 50% reduction in plant dry weight) and resistance ratio (R50/S50) values of resistant (R) and susceptible samples for each province (S) *Echinochloa crus-galli* biotypes to penoxsulam and bispyribac-sodium in dose response experiments

Country	ALS inhibitor					
	Penoxsulam			Bispyribac-sodium		
	NO	ED_{50}	R_{50}/S_{50}	NO	ED_{50}	R_{50}/S_{50}
Iran	GOL-59	85.68 (6.97)	7.07	GIL-186	23.96 (2.59)	2.83
	GOL-148*	12.12 (1.69)	1.00	GIL-160*	8.47 (0.63)	1.00
	-	-	-	GOL-62	46.14 (3.56)	4.32
	-	-	-	GOL-170	32.78 (4.03)	3.07
	-	-	-	GOL-47	25.54 (3.21)	2.40
	-	-	-	GOL-3	42.81 (5.58)	4.00
	-	-	-	GOL-148	25.27 (2.48)	2.36
	-	-	-	GOL-143	38.49 (3.30)	3.60
	-	-	-	GOL-153	31.68 (2.28)	2.96
	-	-	-	GOL-185	23.63 (1.01)	2.21
	-	-	-	GOL-2	49.22 (3.78)	4.60
	-	-	-	GOL-82	38.52 (2.74)	3.60
	-	-	-	GOL-65	85.41 (7.31)	8.00
	-	-	-	GOL-150	34.33 (2.57)	3.21
	-	-	-	GOL-36	38.02 (3.51)	3.56
	-	-	-	GOL-7	33.80 (4.14)	3.16
	-	-	-	GOL-4	29.21 (1.72)	2.73
	-	-	-	GOL-191	37.75 (2.77)	3.53
	-	-	-	GOL-177	31.84 (5.26)	2.98
	-	-	-	GOL-26	35.01 (3.22)	3.27
	-	-	-	GOL-41*	10.69 (0.95)	1.00
	-	-	-	MAZ-127	32.12 (3.54)	2.26
	-	-	-	MAZ-193*	14.19 (1.11)	1.00
Turkey	COR-1	60.76 (5.48)	3.74	COR-1	51.29 (3.90)	2.82
	COR-4	54.67 (4.54)	3.37	COR-4	49.71 (4.68)	2.74
	COR-7	37.31 (2.59)	2.30	COR-16	44.98 (4.58)	2.48
	COR-16	41.34 (4.27)	2.55	COR-43	39.84 (3.69)	2.20
	COR-9*	16.23 (0.95)	1.00	COR-49	44.85 (3.82)	2.47
	SAM-55	48.48 (4.89)	2.66	COR-58	40.08 (3.91)	2.21
	SAM-60	48.92 (3.66)	2.69	COR-7	52.14 (3.16)	2.87
	SAM-74	61.12 (9.24)	3.36	COR-33	52.35 (3.62)	2.88
	SAM-92	43.90 (3.98)	2.41	COR-38	40.65 (4.44)	2.24
	SAM-6	44.14 (3.00)	2.43	COR-75*	18.16 *0.92)	1.00
	SAM-9	48.62 (3.15)	2.67	SAM-32	53.31 (5.19)	2.81
	SAM-21	42.27 (4.60)	2.32	SAM-35	46.01 (4.24)	2.42
	SAM-32	44.62 (4.63)	2.45	SAM-48	38.86 (4.22)	2.05
	SAM-35	42.24 (3.91)	2.32	SAM-55	48.47 (2.94)	2.55
	SAM-48	57.78 (3.54)	3.18	SAM-60	40.82 (3.17)	2.15
	SAM-12*	18.19 (1.09)	1.00	SAM-74	70.31 (4.10)	3.70
	-	-	-	SAM-92	43.01 (3.61)	2.27
	-	-	-	SAM-6	40.92 (4.58)	2.16
	-	-	-	SAM-9	40.87 (3.75)	2.15
	-	-	-	SAM-12	42.36 (3.01)	2.23
	-	-	-	SAM-19	42.40 (2.98)	2.23
	-	-	-	SAM-21	41.27 (3.03)	2.17
	-	-	-	SAM-28*	18.97 (1.56)	1.00
	-	-	-	SIN-20	37.26 (3.01)	2.20
	-	-	-	SIN-27	37.52 (2.58)	2.22
	-	-	-	SIN-1*	16.89 (0.84)	1.00

Values in parentheses are standard errors. *Sensitive samples for each province. GOL = Golestan, GIL = Gilan, MAZ = Mazandaran, COR = Corum, SAM = Samsun, SIN = Sinop.

these herbicides are not registered in Iran. Most of the resistant biotypes have been observed in Corum and Golestan regions, with continuous rice cultivation, without any crop rotation. The present cropping pattern is a significant risk for rice cultivation and will contribute to evolution of further weed resistance in the future. In case of multiple resistances to herbicides and due to the limited number of alternative herbicides, the cost of preventive operation would be significantly less than fighting against evolution of resistance. When weed

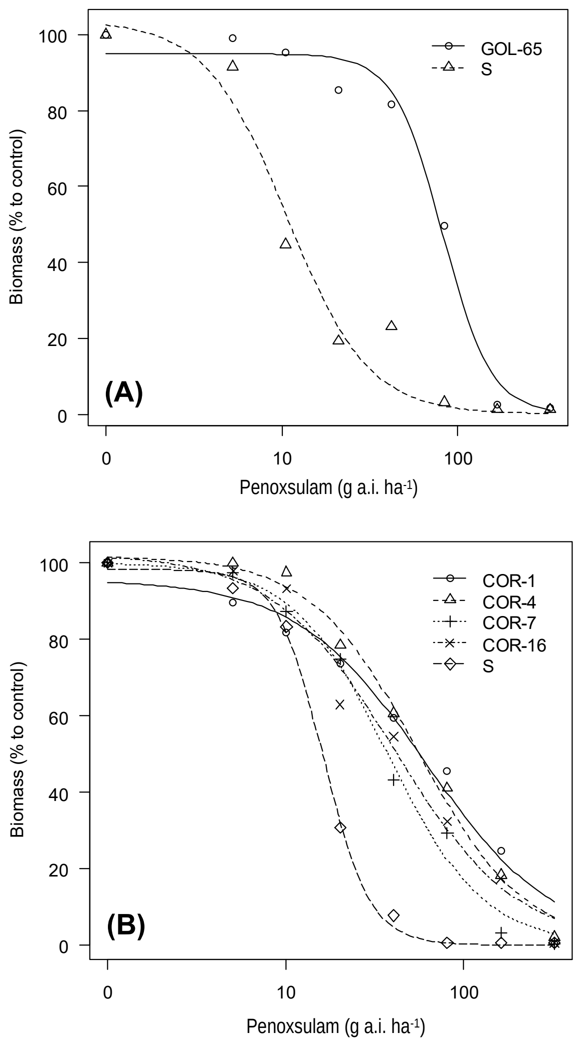
Table 2 - Estimate of ED50 (herbicide rate required to cause 50% reduction in plant dry weight) and resistance ratio (R50/S50) values of resistant (R) and susceptible samples for each province (S) *Echinochloa crus-galli* biotypes to cyhalofop-butyl in dose response experiments

Country	ACCase inhibitor		
	cyhalofop-butyl		
	NO	ED_{50}	R/S
Turkey	COR-1	471.4 (40.06)	5.37
	COR-4	415.5 (46.65)	4.73
	COR-7	271.4 (32.18)	3.01
	COR-16	399.3 (49.76)	4.55
	COR-33	276.0 (19.61)	3.14
	COR-43	295.2 (29.90)	3.36
	COR-9	217.9 (38.02)	2.48
	COR-38	228.3 (25.91)	2.60
	COR-49	193.2 (15.83)	2.20
	COR-58	226.6 (11.51)	2.58
	COR-75	197.5 (19.33)	2.25
	COR-26*	87.8 (7.20)	1.00
	SAM-6	298.7 (25.41)	2.61
	SAM-74	291.1 (33.07)	2.55
	SAM-92	291.1 (24.88)	2.55
	SAM-12*	114.3 (6.58)	1.00
	SIN-20	290.6 (21.52)	2.35
	SIN-27	330.6 (21.23)	2.67
	SIN-1	292.7 (23.87)	2.37
	SIN-7*	123.7 (8.64)	1.00

Values in parentheses are standard errors. * Sensitive samples for each province. COR = Corum, SAM = Samsun, SIN = Sinop.

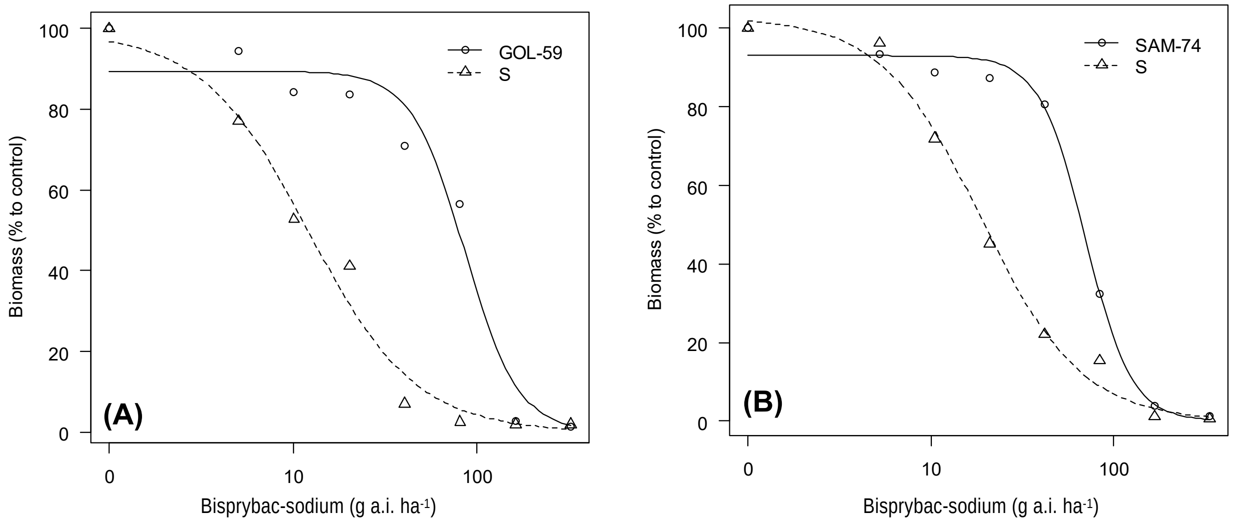
control in first year is poor, farmers will incur great costs to dispel resistance, but if alternate affordable herbicide is available, resistance control cost would reduce (Peterson, 1999).

Rubin (1997) defined cross-resistance as a case where a weed biotype is resistant to two or more herbicides acting at the same primary site of action



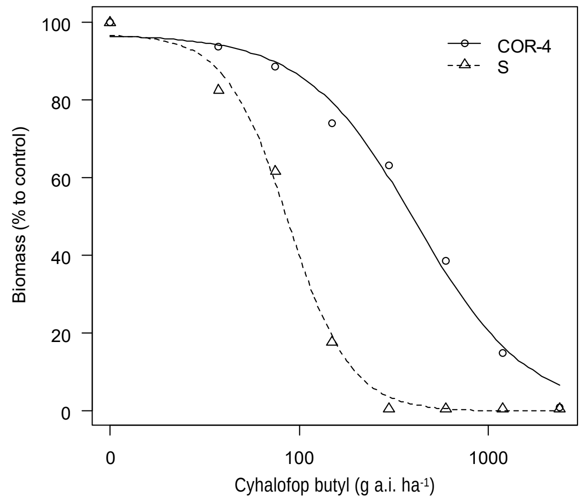
Each data point represents the average of the two experiments.

Figure 1 - Dose-response pattern of penoxsulam on different *Echinochloa crus-galli* biotypes which is collected from a) Iran and b) Turkey in 2013 (COR-9 susceptible sample).



Each data point represents the average of the two experiments.

Figure 2 - Dose-response pattern of bispyribac-sodium *Echinochloa crus-galli* biotypes (resistant and susceptible) which is collected from (A) Iran and (B) Turkey in 2013 (GOL-41 and SAM-28, susceptible samples).



Each data point represents the average of the two experiments.

Figure 3 - Dose-response pattern of cyhalofop-butyl on resistant and susceptible *Echinochloa crus-galli* biotypes which is collected from Turkey in 2013 (COR-26 susceptible sample).

and multiple resistances, when a biotype is resistant to two or more herbicides functioning at different sites. ALS inhibitors such as bensulfuron-methyl, cinosulfuron and ethoxysulfuron are used in some specified areas, but there is no history of bispyribac-sodium application to rice fields in Iran. Apparently, bispyribac-sodium resistance outbreak in *E. crus-galli* in Golestan province, Iran may be considered as cross-resistance. Resistant genes exist in the plant before selection (the initial frequency of resistant genes). Hence, if the initial frequency of resistant gene is high, resistance development will be fast. Also, there are numerous reports of natural resistance in weeds to ALS inhibitors (Preston et al, 2006).

Results also showed that, in Iran, especially in Golestan province, a greater percentage of samples were resistant to both ALS inhibitors. Northern provinces of Sinop in Turkey possessed a less ratio of weeds resistant to both ALS and ACCase inhibitors than Samsun and Corum. Since cyhalofop herbicide has been used consecutively in turkey, resistance to this herbicide is within logic. This is also the case for lack of cyhalofop- resistance in Iran, as this herbicide has not been used in the country. Resistance to ALS inhibitors bispyribac sodium and penoxsulam in Iran may be justified with the fact that there is history of some ALS herbicides being continuously applied in crops cultivated in rotation with rice, which has eventually led to evolution of cross-resistance to the studied herbicides.

Ideally, farmers should apply integrated weed management system (IWM) to avoid resistance outbreak, and multiple-resistance in weed species of poaceae is a major drive for adoption of IWM

Table 3 - Resistance status of the biotypes collected from turkey

Biotype	Resistance/cross-resistance	Multiple resistance
COR-1	Bispyribac-sodium×penoxsulam	Bispyribac-sodium×penoxsulam×cyhalofop-butyl
COR-4	Bispyribac-sodium×penoxsulam	Bispyribac-sodium×penoxsulam×cyhalofop-butyl
COR-7	Bispyribac-sodium×penoxsulam	Bispyribac-sodium×penoxsulam×cyhalofop-butyl
COR-9	Penoxsulam	Cyhalofop-butyl×penoxsulam
COR-16	Bispyribac-sodium×penoxsulam	Bispyribac-sodium×penoxsulam×cyhalofop-butyl
COR-33	Bispyribac-sodium	Cyhalofop-butyl×bispyribac-sodium
COR-38	Bispyribac-sodium	Cyhalofop-butyl×bispyribac-sodium
COR-43	Bispyribac-sodium	Cyhalofop-butyl×bispyribac-sodium
COR-49	Bispyribac-sodium	Cyhalofop-butyl×bispyribac-sodium
COR-58	Bispyribac-sodium	Cyhalofop-butyl×bispyribac-sodium
COR-75	Cyhalofop-butyl	----
SAM-6	Bispyribac-sodium×penoxsulam	Bispyribac-sodium×penoxsulam×cyhalofop-butyl
SAM-9	Bispyribac-sodium×penoxsulam	----
SAM-12	Bispyribac-sodium	----
SAM-19	Bispyribac-sodium	----
SAM-21	Bispyribac-sodium×penoxsulam	----
SAM-32	Bispyribac-sodium×penoxsulam	----
SAM-35	Bispyribac-sodium×penoxsulam	----
SAM-48	Bispyribac-sodium×penoxsulam	----
SAM-55	Bispyribac-sodium×penoxsulam	----
SAM-60	Bispyribac-sodium×penoxsulam	----
SAM-74	Bispyribac-sodium×penoxsulam	Bispyribac-sodium×penoxsulam×cyhalofop-butyl
SAM-92	Bispyribac-sodium×penoxsulam	Bispyribac-sodium×penoxsulam×cyhalofop-butyl
SIN-1	Cyhalofop-butyl	----
SIN-20	Bispyribac-sodium	Cyhalofop-butyl×bispyribac-sodium
SIN-27	Bispyribac-sodium	Cyhalofop-butyl×bispyribac-sodium

(Norsworthy et al., 2012). Currently, in many regions, excessive use of pesticides are not favoured as before (Frisvold, 2019) and old methods are being revived such as crop rotation (Hunt et al., 2017), sowing date selection (Valverde et al., 2000), adopting agricultural operations like ploughing (Furlan et al., 2020), gene dispersal reduction (Yan et al., 2017), using alternate herbicides with different modes of action (Marochi et al., 2018), application of mixed herbicides (Abbas et al., 2018), and combination of herbicides with other agricultural and mechanical practices (IWM) (Adeux et al., 2017). These methods are effective if farmers accept changes and revive long term farm economy, for which they will need further support from governmental and industrial researches and promotion efforts around the world. This may achieve farmers' cooperation and acceptance and raise their awareness towards complications of resistance to herbicides.

4 Conclusions

The rapid expansion of herbicide resistance in both countries indicate the necessity of adopting integrated weed management practices to hinder the further evolution of resistance in future.

5 Contributions

KH: data curation, analysis, writing of the manuscript. HM: supervision, editing of the manuscript.

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