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Full Length Article

First Report of Herbicide-Resistant *Echinochloa colona* from the Rice Fields of Punjab, Pakistan

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Abstract

This study used rapid resistance in-season quick test (RISQ) for confirming bispyribac-sodium resistance in jungle rice (*Echinochloa colona* (L.) Link.), which is troublesome invasive weed of rice fields worldwide. Field survey was conducted to collect bispyribac-sodium resistance-suspected seeds from various rice fields located in the Punjab province of Pakistan during the year 2020. The RISQ test and classical assay (dose-response assay) were performed to check bispyribac-sodium resistance in *E. colona*. The results of RISQ test revealed that *E. colona* in surveyed locations had different resistance levels to bispyribac-sodium with a resistance index (RI) ranging from 2.52 to 6.70. Populations named EC-5 and EC-7 showed the highest resistance with RI of 6.70. This represented the first case of bispyribac-sodium resistant *E. colona* in the Indian subcontinent. The results of RISQ test were consistent with conventional dose-response assay. Further, the efficacy of alternate herbicides including pendimethalin, acetochlor, ethoxysulfuron-ethyl and penoxuslam was evaluated against *E. colona*. Findings revealed effective control of bispyribac-sodium resistant *E. colona* with alternate herbicides. The RISQ test used in the current study will help in early detection of resistance in *E. colona* for correct in season application of post-emergence herbicides to reduce rice yield losses and spread of resistant weed seeds.

Keywords: Herbicide resistance; Jungle rice; ALS inhibitor; Resistance management; Cross resistance; Rice weed
Abbreviations: RISQ, Resistance In-Season Quick Test; RI, Resistance Index; ALS, Acetolactate Synthase; EPSP, 5-enolpyruvylshikimate 3-phosphate; ALS, acetolactate synthase; PS, Photosystem; FYM, Farmyard manure; LD_{50}, lethal dose to 50%

Introduction

Rice (*Oryza sativa* L.) is third most widely grown crop after wheat and cotton in Pakistan and country is the fifth largest exporter of basmati rice in the world (Chauhan *et al.* 2017; Matloob *et al.* 2022). Rice contributes about 17% of the total cereal production in the country and 67% to total basmati rice production in the world (Mann *et al.* 2016; Matloob *et al.* 2022). The aromatic basmati rice produced in Pakistan is famous in the world due to its aroma and cooking quality and is a major export commodity of the country (Government of Pakistan 2020) and its straw is also used for by-products (Singhajutha *et al.* 2020). The jungle rice (*Echinochloa colona* (L.) Link) is a monocot weed that belongs to the *Poaceae* family and is damaging in subtropical and tropical regions in field crops such as maize (*Zea mays* L.), rice (*Oryza sativa* L.), and vegetables, especially in Asia, Africa, and Australia (Kraehmer *et al.* 2016; Peerzada *et al.* 2016). It is a fast-spreading invasive weed in America and Malaysia and in wetlands of Australia (Bajwa *et al.* 2015). High seed production potential, low seed dormancy, multiple propagation ways and adaptability to diversified ecological conditions have made it successful and dominant in different agri-ecosystems (Peerzada *et al.* 2016). In Pakistan, *E. colona* is among the top troublesome and widespread weeds (Shad and Siddiqui 1996). In India, it is the most harmful and yield decreasing weed in summer crops, and second abundant weed in direct seeded rice (Mahajan *et al.* 2013).

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Use of herbicides is the major method for the control of *E. colona* because of herbicides' high efficacy, low cost and easy application (Singh *et al.* 2016; Phumkhachorn and Rattanachaikunsopon 2020). Due to current water crises and high labor cost, farmers are practicing direct seeded rice that made weed control more important and the use of herbicides has increased (Chauhan *et al.* 2015; Jabran and Chauhan 2015). Different herbicides including acetochlor, butachlor, ethoxysulfuron-ethyl, oxadiargyl, pendimethalin, bisyaribac-sodium and azimsulfuron have been efficaciously practiced for controlling *E. colona* in rice fields (Jabran and Chauhan 2015; Jabran *et al.* 2012). However, resistance evolution in *E. colona* against several herbicide mode of actions (MOA) has been reported in different parts of world. For example, 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase inhibitor, synthetic auxins, ACCase inhibitors, ALS inhibitors, photosystem II(PSII) inhibitors in various countries worldwide (Heap 2023). Evolution of resistance in *E. colona* against acetolactate synthase (ALS) inhibitors due to continuous and widespread use of these herbicides has been reported in various countries (Heap 2023). Furthermore, multiple resistances (2 or 3 sites of actions) in *E. colona* against ALS, cellulase, EPSP and PII inhibitors were evaluated in Costa Rica, United States (Arkansas) and Venezuela (Heap 2023).

Currently, there is no confirmed report of resistant *E. colona* in major rice producing countries including China, India, Bangladesh, Japan, Philippines, and Pakistan. Nevertheless, in the last few years, rice farmers in Pakistan have been facing increasing problem of uncontrolled *E. colona* plants in rice fields after it is sprayed with bispyribac-sodium. Recently, the acetyl-CoA carboxylase inhibitor resistance in *Phalaris minor* as first resistance case in Pakistan was reported in 2016 (Abbas *et al.* 2017). Early confirmation and management of resistant *E. colona* is essential to sustain rice production and prevent yield losses (Burgos 2015). Alternative herbicides if applied with sufficient time intervals can play an important role in controlling bispyribac-sodium resistant *E. colona*, thus tumbling the rice yield losses due to resistance issue (Vidotto *et al.* 2020). In addition, more herbicides with a different mode of actions should be available to be used in rotation or mixtures, it will help reduce selection pressure that may lead to delayed resistance evolution (Beckie and Reboud 2009; Burgos 2015). Among resistance confirmation methods the classical dose response assay is the most suitable and reliable method to confirm resistance and assess resistance index (Burgos *et al.* 2013; Burgos 2015). Although this method is more reliable, it is time-consuming and cannot be used to evaluate herbicide resistance during the same growing season. Hence late confirmation of resistance may cause reduction in crop yield in current season, spread of resistant weed seeds and wrong use of herbicides.

The Syngenta Resistance In-Season Quick (RISQ) test is a method for confirming resistance in range of grasses and broadleaf weeds to post emergence herbicides over a short period of time (Kaundun *et al.* 2011). This test is extremely useful for weed scientists and farmers to confirm resistance in the current growing season without the collection of seeds at maturity and dealing with seed dormancy issues (Kaundun 2021). Early resistance detection creates an opportunity to apply intermediate correct dose of some alternate herbicide to control resistant weeds in the current season to check crop yield losses. The study was conducted with the following objectives: (1) to confirm bispyribac-sodium (ALS inhibitor) resistance in *E. colona* populations from rice fields of Punjab region of Pakistan (2) to use RISQ test to evaluate resistance in *E. colona* (3) to evaluate the efficacy of alternate herbicides to control bispyribac-sodium resistant *E. colona* and to check potential cross resistance.

Materials and Methods

Survey to collect *E. colona* seeds

Seed samples of *E. colona* putative to bispyribac-sodium resistance were collected from eight different areas of Punjab, Pakistan during the year 2020. Recently, farmers faced difficulties in controlling *E. colona* with bispyribac-sodium in rice fields of these areas. The field history of cropping pattern, herbicides use and location of surveyed in fields is given in Table 1. *E. colona* seed heads that survived after bispyribac-sodium application during the season were collected from rice fields. We selected eight different rice fields from each location (Table 1), 30 random plants from each field were considered for seed collection (Burgos 2015). The collected seeds were tagged as representative samples for each location. The seeds were sun-dried and kept in paper bags under dry condition at room temperature. Bispyribac-sodium susceptible *E. colona* seeds were collected from the field having no use of bispyribac-sodium and had known history of efficient control, for comparison with resistance suspected populations.

Dose-response assay for resistance confirmation

We performed repeated bioassay under greenhouse conditions to quantify the resistance profile of *E. colona*. The greenhouse is located at about 32.13º N latitude, 72.68º E longitude, and at an altitude of 190 m in College of Agriculture, University of Sargodha, Sargodha, Pakistan. Fifteen seeds were sown in each pot (15×12×5 cm) filled with herbicide residue free soil and covered with equal quantity of fine soil. Soil was mixed with well decomposed weed seed free farmyard manure (2:1, w/w). We applied distilled water when needed throughout the experimental duration. In greenhouse, the minimum and maximum temperature recorded were 30 and 36ºC, photoperiod was 14-h, and humidity ranged from 35 to 70%.

For a dose–response test, pots were set in a randomized complete block design. The treatments were replicated four times, and each replicate contained 15 seeds. Bispyribac-sodium (Nominee®$_{100}$SC, Crop Care and Environmental Sciences, Pakistan) was sprayed when weed plants were at 3-4 true leaves growth stage. Six doses of the herbicides were used including 0, 0.125X, 0.25X, 0.5X, 1X and 2X (Burgos, 2015; Burgos *et al.*, 2013). Recommended field rate (X) was 125 mL ha^{-1} (30 g a.i. ha^{-1}) of product with active ingredient (bispyribac-sodium) 9.7% w/w. The herbicide spray was done with a CO_2 pressurized sprayer usingTeeJet8003VS nozzle at 30 psi pressure and the approximate volume of spray was 186 L per ha. Mortality percentage was based on the number of weed plants killed by herbicide to total number of plants before herbicide exposure (15 plants per pot were maintained). The weed biomass was recorded twenty-eight days after spray.

RISQ test for resistance confirmation

For agar media preparation, agar powder was added in ultra-pure water at 1.2% w/v ratio and placed in autoclave. Respective herbicide doses were added to agar solution when it was cooled to 55°C and gently mixed for 2 min. The petri plates without herbicide were considered as control. Petri plates were arranged under completely randomized design with four replications for each treatment. For seedling preparation, the collected seed samples of *E. colona* putative to bispyribac-sodium resistance were sown in small pots under greenhouse conditions following the same procedure mentioned in dose-response assay. Seedling at one to three leave stages were carefully uprooted from pots and washed well with water and removed extra moisture using filter paper. The seedlings were placed horizontally in agar media containing different doses of bispyribac-sodium herbicide, with roots fully dipped inside the agar media. Forty plants of each biotype were tested against each herbicide treatments with 8 plants per petri plate. Petri plates were covered with lids and placed in incubator with temperature 30±2°C for day and 25±2°C for night with 12 h photoperiod. Data regarding mortality based on number of seedlings killed to the total number of seedling transplanted for each treatment was calculated. LD50 and RI index was calculated for all the tested biotypes.

Efficacy of alternate herbicides

Resistance was confirmed in first bioassay, in this study the population with the highest resistance index (EC-5) was used to check cross-resistance and control efficacy of other herbicides using dose response assay. Experimental conditions and procedures were as explained in the first resistance confirmation bioassay. Five alternate herbicides including pendimethalin (Stomp 330 EC) at 1320 g a.i. ha^{-1} and acetochlor (Harness®) at 1050 g a.i. ha^{-1} as pre-emergence and fenoxaprop-p-ethyl (Puma Super® 750 EW, Bayer crop science, Pakistan) at 93.75 g a.i. ha^{-1}, ethoxysulfuron-ethyl (Sunstar Gold 60WG) at 30 g a.i. ha^{-1} and penoxuslam (Granite) at 15 g a.i. ha^{-1} as post-emergence were tested. Pre-emergence herbicides were used before the emergence of weed seeds, while post-emergence herbicides were sprayed at 3–4 leaf stage at six different doses (0, 0.125X, 0.25X, 0.5X, 1X and 2X) using the same procedure used for the resistance confirmation assays. The experiments were repeated, however the results of repeated studies were statistically similar, so, data were pooled before analysis.

Mortality data were subjected to probit analysis by using inverse prediction of logistic 3P in JMP 13 to calculate the herbicide rate necessary to kill 50% of (LD50) of each biotype.

$LD_{50} = c/(1+\exp\{-a(dose-b)\})$

Where a is growth rate, b is an inflection point, c is an asymptote.

Resistance index was determined as the ratio of the LD_{50} of each resistant population and the LD_{50} of the susceptible population (Travlos *et al.* 2011; Burgos *et al.* 2013).

Resistance level = (LD_{50} of resistant population)/(LD_{50} of susceptible population).

Results

Dose-response assay for resistance confirmation

Eight collected *E. colona* populations indicated different levels of resistance against bispyribac-sodium (Table 2). The recommended dose of bispyribac-sodium (30 g a.i. ha^{-1}, 1X) resulted in 100% mortality in the susceptible population, however, the other i.e., the resistance suspected *E. colona* populations showed low mortality (24.21–79.5%) at this dose. The response of *E. colona* populations at 2X showed that 100% mortality occurred in the susceptible population, mortality range for other populations was 38.23–86.54%. Among various accessions, the EC-5 showed a higher resistance with the resistance index of 7.07. The LD_{50} and resistance index of different collected populations were in the range of 0.62–1.84 and 2.42–7.07, respectively (Table 2). Based on LD_{50} and resistance index, each location showed a differential response to bispyrabic-sodium. EC-5 exhibited a higher resistance to bispyrabic-sodium as compared to other accessions.

RISQ test for resistance confirmation

The collected *E. colona* populations tested with RISQ test indicated different levels of resistance for bispyribac-sodium (Table 3, 4; Fig. 1). A 100% mortality was occurred at recommend field dose in susceptible population, however, other resistance suspected *E. colona* populations showed low mortality ranging from 21.23 to 83.18% at 1X. The response of *E. colona* populations at 2X showed that mortality in resistance suspected populations ranged from 41.60 to 96.54%.

Table 1: Districts, locations, and crop history of different *E. colona* populations collected for bispyribac-sodium resistance confirmation

E. colona populations	Districts	Locations	Field history	
			Cropping system	Herbicide use (Years)
EC-1	Mandi Bahauddin	32°19′ N, 73°29′ E	Maize-wheat	>20
EC-2	Mandi Bahauddin	32°31′ N, 73°48′ E	Rice-wheat	>20
EC-3	Faisalabad	31°36′ N, 73°60′ E	Mix cropping	15-18
EC-4	Faisalabad	30°89′ N, 72°85′ E	Mix cropping	15-18
EC-5	Sargodha	32°08′ N, 72°73′ E	Rice-wheat	>20
EC-6	Sargodha	32°29′ N, 73°00′ E	Maize-wheat	12-14
EC-7	Gujranwala	31°94′ N, 74°02′ E	Rice-wheat	>20
EC-8	Gujrat	32°83′ N, 74°00′ E	Rice-wheat	>15
EC-SS	Faisalabad	31°45′ N, 73°00′ E	Non-cropping	1-2

Table 2: Percent mortality, LD_{50} and resistance level of different *E. colona* populations against bispyribac-sodium in dose-response assay

E. colona accessions	Mortality percentage						LD50[a]	Resistance index[b]
	0	0.125X	0.25X	0.5X	1X	2X		
EC-1	0 ± 0.00	6.00 ± 2.15	20.00 ± 3.21	23.00 ± 2.16	54.50 ± 3.41	64.20 ± 1.31	0.96	3.69
EC-2	0± 0.00	2.97 ± 2.34	10.00 ± 3.13	33.40 ± 1.83	72.70 ± 2.36	86.05 ± 3.32	0.63	2.42
EC-3	0 ± 0.00	0.00 ± 0.00	8.10 ± 2.77	19.78 ± 2.77	34.12 ± 2.77	53.40 ± 3.73	1.25	4.80
EC-4	0± 0.00	5.00 ± 2.24	10.10 ± 3.22	36.23 ± 1.28	79.50 ± 2.72	86.54 ± 3.22	0.62	2.38
EC-5	0 ± 0.00	0.00 ± 0.00	4.50 ± 2.87	7.88 ± 3.81	24.21 ± 2.88	40.60 ± 2.87	1.84	7.07
EC-6	0 ± 0.00	0.00 ± 0.00	10.00 ± 2.14	18.10 ± 2.71	60.34 ± 3.61	71.27 ± 2.16	1.12	4.30
EC-7	0 ± 0.00	0.00 ± 0.00	0.00 ± 2.66	8.11 ± 3.06	25.54 ± 2.56	38.23 ± 2.66	1.77	6.80
EC-8	0 ± 0.00	0.00 ± 0.00	22.30 ± 3.42	36.30 ± 1.84	74.29 ± 1.48	79.40 ±1.74	0.69	2.65
EC-SS	0 ± 0.00	20.00 ± 2.88	35.50 ± 1.62	94.00 ± 3.22	100.00 ± 0.02	100.00 ± 0.00	0.26	--

[a]LD_{50} was predicted by using inverse prediction of logistic 3P in JMP 13. [b]RI was calculated by dividing the LD_{50} dose (g a.i. ha^{-1}) of resistant biotype by the LD_{50} dose of susceptible biotype. The data are the means ± standard error

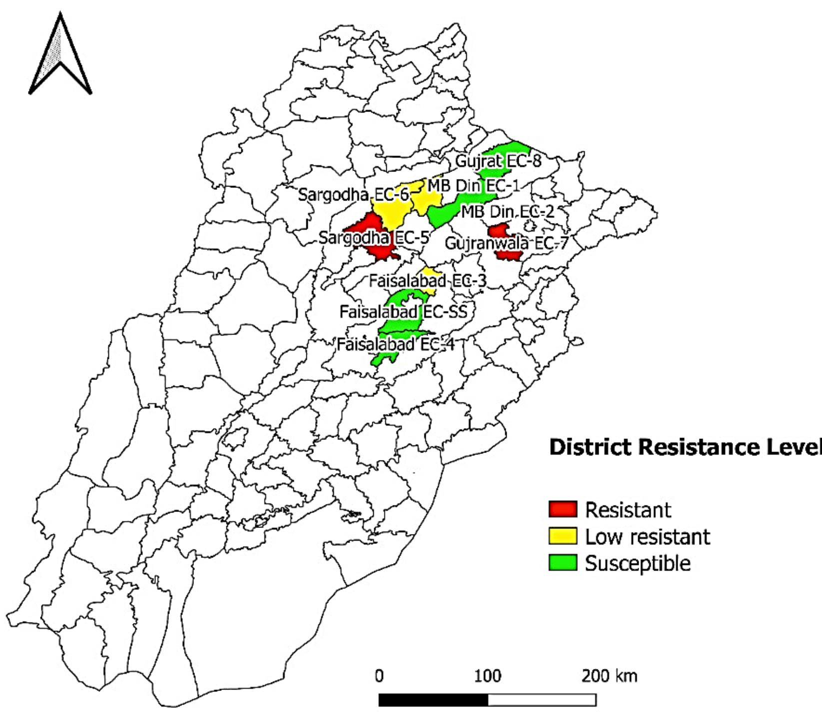

Fig. 1: Locations on map with district and population names showing resistant, mildly resistant and susceptible *E. colona* populations collected for bispyribac-sodium resistance confirmation

Among various accessions, the EC-5 showed a higher resistance with the resistance index of 6.70 in RISQ test. The LD_{50} and resistance of different collected populations were in the range of 0.56–1.81 and 2.07–6.70, respectively (Table 3). Based on LD_{50} and resistance index, each location showed a differential response to bispyrabic-sodium. EC-5 exhibited a higher resistance to bispyrabic- sodium as compared to other accessions in RISQ test.

Table 3: Percent mortality, LD_{50} and resistance level of different *E. colona* populations against bispyribac-sodium in RISQ test

E. colona accessions	Mortality percentage						LD50[a]	Resistance index (RI)[b]
	0	0.125X	0.25X	0.5X	1X	2X		
EC-1	0 ± 0.00	4.00 ± 2.05	18.00 ± 3.22	27 00± 2.56	55.32 ± 3.43	65.00 ± 2.31	0.88	3.41
EC-2	0± 0.00	2.66 ± 2.64	9.34 ± 3.01	32.64 ± 1.68	69.97 ± 2.76	85.05 ± 3.02	0.68	2.52
EC-3	0 ± 0.00	0.00 ± 0.00	7.90 ± 2.78	19.78 ± 2.75	37.12 ± 2.67	59.40 ± 3.03	1.25	4.33
EC-4	0± 0.00	4.53 ± 2.64	9.12 ± 3.01	42.92 ± 1.68	84.22 ± 2.76	96.54 ± 3.02	0.56	2.07
EC-5	0 ± 0.00	0.00 ± 0.00	5.54 ± 2.67	6.98 ± 3.01	21.23 ± 2.58	41.60 ± 2.67	1.81	6.70
EC-6	0 ± 0.00	0.00 ± 0.00	8.43 ± 2.54	20.00 ± 2.75	51.34 ± 3.65	72.27 ± 2.66	1.09	4.03
EC-7	0 ± 0.00	0.00 ± 0.00	2.34 ± 2.67	8.43 ± 3.01	28.18 ± 2.58	43.21 ± 2.67	1.76	6.51
EC-8	0 ± 0.00	0.00 ± 0.00	20.01 ± 3.02	35.70 ± 1.89	73.67 ± 1.68	83.18 ±1.78	0.68	2.74
EC-SS	0 ± 0.00	15.00 ± 2.88	36.25 ± 1.67	93.75 ± 3.23	100.00± 0.00	100.00± 0.00	0.27	--

[a]LD_{50} was predicted by using inverse prediction of logistic 3P in JMP 13. [b]RI was calculated by dividing the LD50 dose (g a.i. ha^{-1}) of resistant biotype by the LD_{50} dose of susceptible biotype. The data are the means ± standard error

Table 4: Efficacy of alternate herbicides to control bispyribac-sodiumresistant *E. colona*

Post emergence herbicides	*E. colona* accessions	Mortality percentage						LD_{50} (X)[a]	Resistance index[b]
		0	0.125X	0.25X	0.5X	1X	2X		
Pendimethalin	Resistant	0 ± 0.00	15.00 ± 1.78	67.23 ± 1.87	90.00 ± 1.98	96.90 ± 2.45	100 ± 0.00	0.22	1.1
	Susceptible	0 ± 0.00	24.12 ± 2.89	68.34 ± 1.86	97.16 ± 2.75	100.00 ± 0.00	100 ± 0.00	0.19	1.00
Acetochlor	Resistant	0 ± 0.00	12.00 ± 2.56	73.32 ± 2.56	92.10 ± 2.45	100.00 ± 0.00	100 ± 0.00	0.21	0.94
	Susceptible	0 ± 0.00	18.23 ± 3.05	77.28 ± 1.45	95.21 ± 1.67	100.00 ± 0.00	100 ± 0.00	0.19	1.00
Fenoxaprop–p–ethyl	Resistant	0 ± 0.00	5.20 ± 3.04	17.34 ± 1.65	35.45 ± 2.64	65.56 ± 3.01	100 ± 0.00	0.89	4.45
	Susceptible	0 ± 0.00	21.33 ± 2.76	40.67 ± 2.05	96.41 ± 1.68	100.00 ± 0.00	100 ± 0.00	0.20	1.00
Ethoxysulfuronethyl	Resistant	0 ± 0.00	12.12 ± 2.79	60.21 ± 2.54	92.23 ± 3.05	100.00 ± 0.00	100 ± 0.00	0.22	1.14
	Susceptible	0 ± 0.00	15.76 ± 1.67	63.12 ± 2.66	92.65 ± 2.76	100.00 ± 0.00	100 ± 0.00	0.21	1.00
Penoxuslam	Resistant	0 ± 0.00	06.65 ± 2.56	63.81 ± 2.56	75.42 ± 2.45	86.00 ± 0.00	100 ± 0.00	0.31	0.94
	Susceptible	0 ± 0.00	18.23 ± 3.05	77.28 ± 1.45	95.21 ± 1.67	100.00 ± 0.00	100 ± 0.00	0.19	1.00

[a]LD_{50} was predicted by using inverse prediction of logistic 3P in JMP 13. [b]RI was calculated by dividing the LD_{50} dose (g a.i. ha^{-1}) of resistant biotype by the LD_{50} dose of susceptible biotype. Data are the means ± standard error

Cross-resistance and potential control options

At recommended dose, susceptible *E. colona* accession showed 100% mortality against all tested herbicides including pendimethalin, acetochlor, fenoxaprop-p-ethyl, ethoxysulfuron ethyl and penoxuslam, whilst response of resistant accessions was different against tested alternate herbicides, and only acetochlor and ethoxysulfuron ethyl gave 100% control at 1X. However, plants treated with 1X of pendimethalin, fenoxaprop–p–ethyl and penoxuslam showed 97, 81 and 86% mortality, respectively. At 2X of the tested herbicides, both resistant and susceptible *E. colona* populations showed 100% mortality (Table 2). Higher LD_{50} (0.50) and RI (4.45) were showed by bispyribac-sodium resistant *E. colona* grass against fenoxaprop-p-ethyl. This shows the susceptibility of bispyribac resistant population to pinoxaden, metribuzin and sulfosulfuron herbicides. Bispyribac-sodium resistant *E. colona* showed a low level of cross-resistance against fenoxaprop-p-ethyl. This may lead to evolution of multiple resistance because of continuous use of fenoxaprop-p-ethyl for many years in Pakistan.

Discussion

Spread of herbicide resistant *E. colona* is among the major sustainability issues for both transplanted and direct seeded rice. This weed has evolved resistance against various herbicide mode actions (Burgos 2015; Heap 2023). However, *E. colona* resistance against bispyrabic sodium has not been reported yet in the Indian subcontinent including the rice fields of Pakistan. As the problem of herbicide resistance in weeds is growing quickly, the herbicide resistance cases are required to be confirmed quickly in order to devise alternative control methods for the herbicide resistant weed populations. Whole plant classical dose response bioassay for resistance confirmation is currently most reliable method for evaluating herbicide resistance (Burgos *et al.* 2013). However, this method is time-consuming and requires at least several months to reach a conclusion.

Hence, as an alternative, various quick tests have been used for in season evaluation of resistance. Among these existing methods, the RISQ test is the most reliable, quick and cheap. This method has been used for confirmation of resistance in various weed species to ACCase inhibitors, ALS inhibitors and glyphosate (Kaundun *et al.* 2011, 2014). Previously, this test has not been used for herbicide resistance confirmation in *E. colona*. In the current study, the comparison of efficacy of classical dose response bioassay and RISQ test to determine herbicide resistance in *E. colona* populations against bispyrabic sodium revealed that RISQ test is equally effective. Results of both the tests were statistically similar, RISQ test discovered as potential quick (gave results in 12 days) resistance evaluation test for *E. colona*. The RISQ test will allow farmers and weed scientists for early detection of herbicide resistance in *E. colona* in the on-going growing season with the advantage to control resistant weeds by spraying proper type and dose of herbicide timely to limit crop yield losses. It will also reduce the labor and cost required for seed collection and to deal with seed dormancy issues faced in classical dose response bioassay (Burgos *et al.* 2013). Further, the development of RISQ test for *E. colona* will assist in quick and accurate confirmation of resistance and help weed scientists in future studies.

Continuous and long-term use of bispyrabic-sodium as early registered herbicide in Pakistan imposed selection pressure to cause resistance evolution (Owen *et al.* 2007). These levels of resistance against bispyribac-sodium have been reported in grassy weeds in other countries (Travlos 2012). Table 1 shows the unique history of each field regarding use of bispyribac-sodium. Bispyribac-sodium was a main herbicide used to control *E. colona* without herbicide rotation. Different levels of resistance in *E. colona* populations might be due to unique selection pressure as each field having a dissimilar history crop and herbicide use (Beckie and Reboud 2009; Travlos 2012). Secondly, different mechanisms of resistance may also be a reason for different level of resistance as reported in other grasses (Maneechote *et al.* 1994). Development of bispyribac-sodium resistance in *E. colona* is the first case of bispyribac resistance in any weed from Pakistan, it is most probably because of prolonged use of bispyribac-sodium herbicide (Vidotto *et al.* 2020). Frequent and prolonged use of bispyribac-sodium can be justified by early registration of this herbicide in Pakistan and farmers trend to use this herbicide alone without rotation as other herbicidal options were not efficient and more expensive to control *E. colona* grass.

All the alternative herbicides used in the study showed good efficacy against bispyribac-sodium resistant *E. colona* except fenoxaprop–p–ethyl that had low level cross resistance. Farmers are using fenoxaprop-p-ethyl in various crops since 1993 in Pakistan. Recently, fenoxaprop-p-ethyl resistance in *P. minor* as first resistance case in Pakistan was also reported in 2016 (Abbas *et al.* 2017). *E. colona* has evolved multiple resistance across various modes of actions including ACCase inhibitors, ALS inhibitors, cellulose synthesis inhibitors and EPSP synthase inhibitors in Colombia, Costa Rica, United States and Venezuela (Heap 2023). Repeated herbicides use and less awareness in farmers regarding resistance evolution might be the major reasons for quick evolution of cross resistance in *E. colona*.

Further, resistant populations are spreading quickly due to the inter change of contaminated (containing resistant weed seeds) rice seeds from farm to farm. Moreover, alternate herbicides, especially ALS inhibitors are more vulnerable to be developed resistance (Chhokar and Sharma 2008). Therefore, to avoid and manage further resistance evolution, integrations of innovative strategies like integration of other crops in rice rotation, use of different herbicides with sufficient intervals, use of herbicides mixture at recommended rate in conjunction with different other agronomic practices for example use of competitive rice varieties, less row spacing, more seed rate and cleanliness to evade seed spread are suggested (Abbas *et al.* 2017; Jabran and Chauhan, 2018; Vidotto *et al.* 2020). Correct quantity and precise spray technique is vital for good control efficacy as low doses may cause hormesis (growth enhancement) in *E. colona* (Abbas *et al.* 2016a). Manual control of weed plants surviving the spray of herbicides before seed production should also be practiced in order to reduce weed seed banks.

Sowing rice at the time that would not coincide with the optimum time of germination of this weed together with reduced tillage practice are effective to decrease the infestation of this weed (Chhokar *et al.* 2007). Allelopathic mulches of crops including sorghum, maize, sunflower and rice can also be used to control weeds (Abbas *et al.* 2016b). Mulches of allelopathic crops were effect to control even herbicide resistant *P. minor*. Rotating rice with other broadleaved crops and multi-cut fodders can also significantly decrease the seeds in soil weed seedbank (Maxwell *et al.* 1990). However, use of crop rotation by replacing rice crop is not possible in a large area because of various constraints including food security as rice is a staple food, the low market of produce for other crops and risk of complete crop collapse. Hence, the instant option is the early detection of resistance and to use the different herbicides in regular change with other agronomic practices for effective control of bispyribac-sodium resistant *E. colona* for sustainable rice production. The RISQ test used in the current study will help in early detection of resistance in *E. colona*. However, future research is required to add more weed species and herbicides, to expand the choice of RISQ test use and reduce the testing time for resistance confirmation for correct application of post-emergence herbicides in current season.

Conclusion

This research used RISQ test for quick and reliable confirmation of bispyribac-sodium resistance in *E. colona* and confirmed the first case of *E. colona* resistance to bispyribac-sodium in the Punjab province of Pakistan. Further, alternate herbicides including pendimethalin, acetochlor and ethoxysulfuron ethyl were found to have good efficacy to manage bispyribac-sodium resistant against *E. colona*. In future, sustainable herbicide resistant management strategies are essential to include in our cropping system to maintain rice production.

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Author Contributions

TA, conceptualization, planning, writing—original draft preparation, writing—review and editing; MAN, writing—review and editing, supervision, resources; KJ, conceptualization, writing—review and editing; AM, AZ and BAK, writing—review and editing; MFS, statistically analyzed the data; NF, writing—review and editing, project administration, data curation. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

Authors do not have any conflict of interest.

Data Availability

All the data generated or analyzed during this study are included in this submission (article).

Ethics Approval

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