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WEED CONTROL IN BARLEY (*HORDEUM VULGARE*) VIA HERBICIDES THAT INHIBIT ALS AND ACCASE WITH INCREASED SEEDING RATE

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SUMMARY

Broad- and narrow-leafed weeds are one of the main challenges that hinder the barley (*Hordeum vulgare* L.) production expansion due to their competitiveness. The latest study aimed to use different methods that have a perpetual effect on weeds in barley fields. A field experiment on barley ensued during crop seasons 2021–2022 and 2022–2023 in the north of Najaf Province, Iraq. Eight combinations and two treatments of the experiment (manual weed control, herbicides, and seeding rate) progressed using a randomized complete block design with 10 replications. Results showed significant differences between treatments and combinations. Saracen, Axial, and weed-free, with a seeding rate of 160 kg ha^{-1}, provided the lowest weed density and the highest weed control efficiency compared with the control (120 kg ha^{-1}). The use of Saracen, Axial, and weed-free, with a seeding rate of 160 kg ha^{-1}, showed the utmost weed control. Based on HPLC analysis of Saracen and Axial residues, the active substances Florasulam and Pinoxaden appeared to be less than the detected level in the grains and straw of barley, which confirms their safety for human and animal consumption. Using herbicides that inhibit acetolactate synthase (ALS) and Acetyl coenzyme-A carboxylase (ACCase), combined with increasing seeding rate, boosts efficient weed control in barley fields.

Keywords: Barley (*H. vulgare* L.), weed population, seed rate, herbicides, weed control, acetolactate synthase (ALS), Acetyl coenzyme-A carboxylase (ACCase)

Key findings: Herbicide use that inhibits the biosynthesis of amino and fatty acids with increasing seeding rate enhanced the efficiency of weed control in barley (*H. vulgare* L.).

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INTRODUCTION

Weeds are one of the chief challenges facing farmers in barley (Hordeum vulgare L.) cultivation in Iraq, as their adverse effects on crop yield characteristics increase competition for water, nutrients, or spatial requirements. The substances secreted from weeds, such as allelochemicals, may cause severe disturbance in root growth, emerging crops' seedling drop, cell cycle, and oxidative activity disruptions (Abbas et al., 2018; Makenova et al., 2023). Weeds also acquire the status of secondary host for many fungal and bacterial pathogens in addition to insects, as previous studies mentioned that crop losses occurred due to weeds and their combined causes (Kanatas et al., 2020).

The dominant weed families affecting barley crops are Poaceae, Brassicaceae, Cyperaceae, and Fabaceae. However, previous studies mentioned up to 50% of losses resulted in weeds spread based on different factors, including weed types, their density, emergence timing, barley density, variety, soil, and environmental factors (Wo´zniak, 2020). Previous reports also stated that the percentage of weed damage on barley crops in the Babylon Province reached 13%, the highest level of damage recorded in the province in 2022 (CSO, 2022). One of the crucial reasons for weed control failure is the dormancy of seeds for long periods and the excessive use of traditional herbicides, repeated for many years. It led to immunity to these herbicides by many types of weeds carrying new environmental and genetic models that make them more tolerant to chemical herbicides' deadly actions (Naeem et al., 2021).

An urgent need to search for economically feasible and environmentally stable alternative methods surfaced that would be ideal in combat the weeds, especially with the emergence of resistance to herbicides by weeds to follow the specialized management method for herbicides with a systemic lethal effect for reducing weeds' harmful effects (Chaudhary et al., 2022).

Multiple techniques have risen in the effective chemical compound manufacture to combat weeds and efficiently discourage physiological processes in weeds. Meanwhile, other applicable agricultural methods continued, such as early barley sowing, crop rotation, seeds-free of weeds, irrigation scheduling, competitive varieties, mechanical control, rotation of selective herbicides use, detection of the emergence of biological differences for weed species, and tillage methods (Meena et al., 2021; Al-Gburi and Al-Gburi, 2023). Thus, the relevant study aimed to evaluate the efficiency of using different approaches with lethal effects on weeds associated with barley crops and detect the herbicide residues in soil and yield.

MATERIALS AND METHODS

Experimental site and procedure

A field experiment ensued during the crop seasons of 2021–2022 and 2022–2023 in the north of Najaf Province, Iraq, with the study site determined by GPS at E° 44.39 and N° 32.30 (Figure 1). The barley cultivar Furat-19 was the best option due to its good germination and adaptation to diverse climates. It is also a registered and accredited cultivar by the Iraqi Ministry of Agriculture. The seeds' mixture with Top Raxil 060 FS pesticide protected them from fungal and bacterial pathogens. The experiment site comprised two fields, F1 and F2, after tillage, and each field contained eight combinations and two treatments with 10 experimental units (80 experimental units). The single experimental unit is about 1 m^2, with the two fields separated by a watering channel, planting field F1 with 120 kg ha^{-1} and field F2 with 160 kg ha^{-1}. Applying (NH_4) $2HPO_4$ (DAP) fertilizer to the soil before planting had 240 kg ha^{-1} average. After the growth of barley plants, urea fertilizer addition was regular at an average of 100 kg ha^{-1} and irrigated the fields when needed.

Identification of weeds in barley crop

Weeds (Poaceae, Brassicaceae, and Fabaceae) identification proceeded phenotypically according to the diagnostic key (Chakravarty, 1976; Barkley, 2004; Shouliang et al., 2006; Zheng-Yi et al., 2010).

Treatments and experiment design

The experiment engaged chemical factors (herbicides), with factors distributed on F1 and F2 fields. The factors are: 1. Control (spraying with water only), 2. Saracen (spraying Saracen herbicide, the active substance is Florasulam 5% SC with the average use of 60 ml ha^{-1} and applied after the emergence of two leaves until the second knot of barley stem), 3. Axial (spraying Axial XL herbicide, the active substance is Pinoxaden 5% EC, using an average of 6 L ha^{-1} and applied after the emergence of two leaves until the second knot of barley stem), 4. Weed-free (comprised of removing weeds manually from the beginning of the experiment until the harvest), and 5. Tween - 0.01% (the Tween mixed with the herbicides at an average of 40 ml ha^{-1}, serving as a diffuser). The randomized complete block design (RCBD) experiment arrangement included 10 replicates for each of the eight combinations, and the total experimental units were 80 in both fields, F1 and F2.

Specific indicators of control

The weed density (weed/1m^2) calculation included a square with a 1-m2 area using the EM-CDTMR visual program, with the numerical density of weeds estimated for each experimental unit according to the method of Meena et al. (2021). The percentage of weed control efficiency in each experimental unit also followed the technique of Meena et al. (2021). The collected indicators in fields 1 and 2 came from the physiological maturity stage of the crop at the beginning of the emergence of spikes and yellowing of leaves. Analysis of herbicide residues in treatments on barley crops ensued. Random samples of barley yield and soil taken from the experimental units of Saracen and Axial treatments reached mixing (Figures 2 and 3). Then, following the procedure by Zhong et al. (2016) helped measure the residues of active substances (Florasulam and Pinoxaden).

Statistical analysis

Data analysis used ANOVA by the Statistical Analysis Software SAS/STAT (2018) by the RCBD (two treatments and eight combinations). Means comparison employed the honestly significant difference (H.S.D). Confirming the findings required one experiment, but only the second experimental data was presentable because no significant differences between each pair of experiments emerged ($P > 0.05$) using the t-test.

RESULTS AND DISCUSSION

Weed density and weed control efficiency

The results showed significant differences between the seeding rate levels with the lowest weed density average and the highest percentage of weed control efficiency, resulting in the 160 kg ha^{-1} seeding rate. It amounted to 19.22 and 78.64, respectively, compared with 24.63 and 72.64 in 120 kg ha^{-1} seeding rate (Tables 1 and 2). Meanwhile, significant differences between the treatments of Saracen, Axial, and weed-free with the 160 kg ha^{-1} seeding rate appeared when it gave the lowest average of weed density at 2.67, 0.0, and 0.0, respectively, and the highest percentage of weed control efficiency at 97.03, 100, and 100, respectively, compared with the control treatment at 120 kg ha^{-1} seeding rate (90.05 and 0.0). The general mean for weed density and the percentage of weed control efficiency were 21.93 and 75.64, respectively.

Many researchers suggested in previous studies that using more than one control method to eliminate weeds obtain superior control and prevent weeds from occurring again in the fields (Hashim et al., 2019; Gyawali et al., 2022). Therefore, the reason for the efficiency of weed control in studied treatments refers to the combination of efficient methods that led to reducing the weed density and increasing the control proportion, as each method has its perpetual effectiveness that differs from the other. The manual uprooting of weeds from the beginning of

Table 1. The effect of studied treatments on weed density (weed/m^2).

Treatments	The amount of seeding in the field		Mean
	120 kg ha^{-1}	160 kg ha^{-1}	
Control	90.05±2.746 a	74.24±0.317 b	82.145±1.981 a
Saracen	6.35±0.825 d	2.67±0.128 de	4.51±0.639 c
Axial	2.13±0.119 de	0 ±0 e	1.065±0.496 dc
Weed free	0.0 ±0 e	0.0 ±0 e	0.0 ±0 d
Mean	24.632±5.528 a	19.227±4.961 b	21.93

Means with different letters = significant differences at $P < 0.05$.

Table 2. The effect of studied treatments on the percentage of weed control efficiency.

Treatments	The amount of seeding in the field		Mean
	120 kg ha^{-1}	160 kg ha^{-1}	
Control	0.0 ±0 h	17.55±0.425 f	8.775±6.978 f
Saracen	92.94±0.351 d	97.03±0.157 ab	94.985±0.866 d
Axial	97.63±0.307 cb	100±0 a	98.815±0.70 bc
Weed free	100±0 a	100±0 a	100±0 a
Mean	72.642±6.419 b	78.645±3.486 a	75.643

Means with different letters = significant differences at $P < 0.05$.

cultivation and its continuation during the growth stages by removing the entire living body of weeds from the field and destroying it was the reason for preventing the spread of weeds through sexual reproduction by seeds or vegetative reproduction by roots (Lodhi et al., 2015; Navish et al., 2017).

The increase in the barley seed quantity is directly proportional to the number of barley plants in the field. Therefore, the rise in barley plants increased the competition for the growth requirements with weeds, negatively affecting weeds not to flower and spread, which reduced their density in the field (Singh and Sarlach, 2022). The Saracen herbicide contains the active substance Florasulam. It has a selective activity to kill the weeds as it inhibits the biosynthesis of amino acids ALS-AHAS, such as Valine, and disrupts metabolism, causing necrosis of weed tissues (Mukherjee, 2020). The Axial herbicide contains the active substance Pinoxaden. It has a selective activity to kill weeds as it inhibits the biosynthesis of fatty acids, cytosol, and plastids, leading to the loss of the integrity of the cell membrane and the death of weed cells (Singh et al., 2017).

Herbicides Saracen and axial residues in soil and grains

The HPLC analysis provided the active substances Florasulam and Pinoxaden in the samples of barley crops at different times. It indicated that the concentration of these substances in the entire plant when applied with herbicide in the field after one day was 296.83 and 88.95 ppm, respectively. After one month, they amounted to 0.104 and 0.073 ppm, respectively. In the soil samples after one day, the rates were 365.42 and 115.82 ppm, respectively, and after one month, at 0.528 and 0.364 ppm, respectively (Tables 3 and 4). However, the substance was undetectable in the plant, seeds, and soil six months later. The failure to detect Florasulam and Pinoxaden (after six months) in the soil allows cultivation without fear of the presence of its harmful residues on the germination of crop seeds or its negative impact on beneficial microorganisms. Similarly, the failure to detect Florasulam and Pinoxaden (after six months) in whole plants and seeds confirms their safety for human and animal consumption after applying Saracen and Axial herbicides in the field.

Table 3. The concentration of Florasulam (ppm) in barley yield.

Sample type	The time of sample analysis (after herbicide application)		
	Herbicide concentration after one day	Herbicide concentration after one month	Herbicide concentration after six months
Whole plant	296.830 ppm	0.104 ppm	N.D.
Seeds	-----	-----	N.D.
Soil	365.420 ppm	0.528 ppm	N.D.

*N.D. = not detected.

Table 4. The concentration of Pinoxaden (ppm) in barley yield.

Sample type	The time of sample analysis (after herbicide application)		
	Herbicide concentration after one day	Herbicide concentration after one month	Herbicide concentration after six months
Whole plant	88.950 ppm	0.073 ppm	N.D.
Seeds	-----	-----	N.D.
Soil	115.825 ppm	0.364 ppm	N.D.

*N.D. = not detected.

Florasulam or Pinoxaden's existence implies that the results of the analysis are similar in the presence of concentrations of active substances after the herbicide application in the field; however, they differed in the rate of the active substances' concentration in the soil or the plant after one day or one month later. A reason may be the difference in the chemical composition of the substance effectivity, which is directly proportional to the metabolic rate in the non-target barley crops treated with Saracen and Axial herbicides. In addition, the active substance Florasulam influences the enzymes forming amino acids. It is unstable in the soil and decays due to weather and irrigation, as its half-life of decomposition is one to four days (Hada et al., 2021).

Florasulam in the barley plant enters several stages, linking through hydroxyl in the aniline ring to nitrogen and then subsequently coupling with glucose, thus reducing its toxic action in barley (Mykhalska et al., 2014; Choudhary et al., 2021). Oloye et al. (2021) showed that the half-life of the decomposition of Pinoxaden in the soil is two to three days under normal conditions, which is consistent with this study's HPLC analysis results. The gradual decline in the concentration of Pinoxaden in barley plants was because it goes through several stages of decomposition and metabolism, followed by the activity of the CYP450 gene capable of converting Pinoxaden into a non-harmful compound in barley crops (Brosnan et al., 2016; Yanniccari et al., 2020).

CONCLUSIONS

Treatments of Saracen, Axial, and weed-free with 160 kg ha^{-1} seeding rate gave the lowest average of weed density and the highest percentage of weed control efficiency. The failure to detect Florasulam and Pinoxaden in the soil allows cultivation without fear of the presence of its harmful residues on the germination of crop seeds or its negative impact on beneficial microorganisms. Likewise, the failure to detect these active substances in whole plants and seeds confirms their safety for human and animal consumption after applying Saracen and Axial herbicides in the field.

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