

Article

Effects of Penoxsulam on Photosynthetic Characteristics and Safety Evaluation of Foxtail Millet

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Abstract: Foxtail millet planting has a long history and profound role in agricultural civilization. However, weeds have become one of the obstacles restricting the development of the foxtail millet industry. Penoxsulam, as an early post-emergence herbicide for controlling gramineous weeds in paddy fields, is effective for some broadleaf weeds. In this study, six different doses (CK, 0.5X, 1X, 2X, 3X, 4X) of penoxsulam were sprayed at the 3–5 leaf stage of the conventional variety Jingu 21 to study its effect on the growth and development of foxtail millet, in order to screen out the appropriate spraying concentration. The main results are as follows: Within 15 days after spraying penoxsulam, the plant height and leaf area of foxtail millet decreased with the increase in spraying dose, and gradually recovered 15–25 days after spraying, but there were still significant differences compared with CK. The photosynthetic pigment content, net photosynthetic rate (P_n), transpiration rate (T_r), stomatal conductance (G_s), the maximum photochemical efficiency (F_v/F_m), photosynthetic system II actual photochemical efficiency ($Y(II)$), and photochemical quenching coefficient (q_P) of foxtail millet decreased with an increase in the penoxsulam spraying dose, while the intercellular CO_2 concentration (C_i) and non-photochemical quenching coefficient (NPQ) showed an upward trend. There was almost no significant difference in each index between the spraying dose of 0.5X and 1X and CK, but the photosynthesis of foxtail millet leaves was still significantly inhibited under a spraying dose of 3X and 4X. Penoxsulam had certain growth-inhibiting effects on *Echinochloa crus-galli* (L.) Beauv. (*E. crus-galli*), *Digitaria sanguinalis* (L.) Scop. (*D. sanguinalis*), *Chenopodium album* L. (*C. album*), and *Amaranthus retroflexus* L. (*A. retroflexus*) which increased as the spraying dosage increased. Our study found that spraying dose groups of 0.5X and 1X penoxsulam were safe for foxtail millet growth and could be used to control gramineous weeds in fields. Other spraying doses are not recommended in the field due to their serious phytotoxicity to foxtail millet, which provides a new measure for weed control in foxtail millet fields.

Keywords: penoxsulam; foxtail millet; agronomic traits; chlorophyll fluorescence parameters; photosynthetic characteristics



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1. Introduction

Foxtail millet [*Setaria italica* (L.) Beauv] is an annual herb plant. It is mainly distributed in arid and semi-arid areas of northern China [1]. The total planting area of foxtail millet in China is approximately 1,500,000 hectares. Its useful characteristics include drought and barrenness tolerance, salt tolerance, high water use efficiency, wide adaptability, strong yield stability, and an ability to grow in poor soil conditions [2,3]. Foxtail millet grains are particularly rich in fiber, starch, protein, and vitamins and have a high iron content [4,5].

Due to its nutritional and health benefits [6], the demand for foxtail millet is high. For these reasons, foxtail millet plays an important role in the agricultural development of Shanxi Province, China—especially in the cultivation of crops rated “special” and “excellent” [7]. In Shanxi Province, the planting area of foxtail millet is ~200,000 hectares, of which Jingu 21 has a planting area of ~100,000 hectares. However, the problems of foxtail millet weeding have seriously restricted the development of foxtail millet production [8].

Due to their rapid growth and huge soil seed bank [9], weeds cause serious harm to farmland. Their growth can reduce the available moisture for crops and impact their growth, and adversely affect the airflow and available light of farmland [10,11]. A foxtail millet field can contain many different types of weeds, and the community structure is complex. The main gramineous weeds are *Echinochloa crus-galli* (L.) Beauv., *Digitaria sanguinalis* (L.) Scop., *Setaria viridis* (L.) Beauv., and *Eleusine indica* (L.) Gaertn. The main broad-leaved weeds include *Chenopodium album* L., *Amaranthus retroflexus* L., *Portulaca oleracea* L., and *Convolvulus arvensis* L. The growth of weeds reduces crop yields by 30–90% [12]. Therefore, during crop production, weeding is a crucial process. At present, the main weeding methods in foxtail millet fields are manual intertillage weeding, mechanical intertillage weeding, and chemical weeding. Foxtail millet is drought- and barrenness-resistant, so it is mostly planted in mountainous dry land in the north. Although mechanical intertillage weeding is highly efficient, the machines are not flexible enough to remove weeds that grow among crop plants, so the technique is generally not used in mountainous areas. In contrast, manual intertillage weeding, which uses simple technology, is highly targeted, clean, and thorough. However, the process is time-consuming, labor-intensive, and inefficient. Because chemical weeding is a convenient, effective, and reliable way to control weeds [13,14], it plays a very important role in weeding. Because they are non-toxic to crops [15], selective herbicides are often used to control weeds during crop production. However, foxtail millet is highly sensitive to herbicides, and there are only seven kinds of herbicides registered in the field: prometryn, sethoxydim, bromoxynil octanoate, monosulfuron, dimethoxyfop-ethyl, 2,4-D isooctyl ester, and 2-Methyl-4-chlorophenoxyacetic acid isooctyl ester. There are currently few studies that focus on developing new herbicides for use in foxtail millet fields. Most of the herbicides used in foxtail millet fields draw lessons from those of other crops, especially those of other wheat crops or gramineous crops, and they easily cause phytotoxicity in foxtail millet.

Herbicides have been proven to be an effective method of controlling weed densities, reducing farmer workload in intensive cropping systems and increasing labor productivity [16]. Herbicides generally target physiological proteins directly involved in plant growth. Many commercial herbicides target the enzyme acetolactate synthase (ALS) and are called sulfonylurea herbicides [17]. These herbicides act by disrupting ALS functions, which is one of the key enzymes involved in the biosynthesis of branched-chain amino acids, such as leucine (Leu), isoleucine (Ile), and valine (Val) [18–20]. ALS inhibitor herbicides can lead to the accumulation of the reaction substrate, 2-ketobutyric acid, and its transamination product, 2-aminobutyrate. These two substances affect the key steps of photosynthesis and interrupt the transport of photosynthetic products, killing the plant [21].

Although the target of action is acetolactate synthase (ALS), the secondary effects of herbicides, such as damage to the photosynthetic system [22] and obstruction of the transportation of photosynthetic products, can also inhibit plant growth and lead to plant death [23]. The research of Yuan et al. [24] showed that the maximum light energy utilization rate (Fv/Fm), apparent electron transfer rate (ETR), and photochemical quenching coefficient (qP) of *Radix Isatidis* decreased obviously at the recommended dose of sigma broad. Feng et al. [25] studied how acetochlor destroyed the photosynthetic system II (PSII) of maize and affected its photosynthesis.

Penoxsulam, an ALS inhibitor, is one of the most important herbicides in rice production and has been widely used in China since 2008 [26]. It is absorbed by stems, leaves, young buds, and roots, and transmitted to the meristem via the xylem and phloem. After 7–14 days of treatment, the apical buds become red and necrotic, and the plants die in

2–4 weeks [27]. Penoxsulam effectively controls *Echinochloa crus-galli*, *Leptochloa chinensis*, and annual *Cyperaceae* weeds, and it is effective against many broad-leaved weeds [28]. At present, the research on penoxsulam mainly focuses on its effect on rice safety and weed control in paddy fields [29,30].

There are few studies on the photosynthetic damage of herbicides to foxtail millet and its safety evaluation. Penoxsulam can effectively control gramineous weeds such as barnyard grass and many broad-leaved weeds, considering the problem of many kinds of weeds and few special herbicides in grain fields; at the same time, there are few reports on related research in penoxsulam in foxtail millet fields. Therefore, it is necessary to study the photosynthetic characteristics and safety evaluation of foxtail millet treated with penoxsulam. In this study based in Shanxi Province, we used the widely cultivated variety “Jingu 21” as the research object, sprayed with different doses of penoxsulam. The main purpose of this study was to study the effects of penoxsulam on the photosynthetic characteristics of Jingu 21 and its safety evaluation, to screen out the safe penoxsulam concentration for Jingu 21, and to formulate a new measure for weed control in foxtail millet fields.

2. Materials and Methods

2.1. Test Materials

The foxtail millet variety, Jingu 21, was provided by Shanxi Academy of Agricultural Sciences. The test agent used in all experiments was 2.5% penoxsulam dispersible oil suspension (active ingredient: 25 g/L, Dow Agricultural Science and Technology Co., Ltd., Nantong, China). Production date: 6 January 2020, batch number: 202001060120200307G.

2.2. Experimental Design

On 12 May 2021, the field experiment was carried out at the key experimental station (37°42' N and 112°58' E). The study area has a temperate continental climate, with an average annual precipitation of 462.9 mm and an average annual temperature of 9.9 °C. The soil was loam, and its basic physical and chemical properties were total nitrogen 1.04 g/kg, total phosphorus 1.12 g/kg, total potassium 18.75 g/kg, available potassium 291.09 g/kg, available phosphorus 23.62 g/kg, alkaline hydrolyzed nitrogen 51.84 g/kg, organic matter 23.36 g/kg, and pH 8.2. During the whole growth period of the foxtail millet, conventional field management (no manual weeding) was carried out according to local ecological conditions. A randomized complete block design was used in the experiment. Different doses of penoxsulam (Table 1) were sprayed at the 3–5 leaf stage of the foxtail millet, and the control was sprayed with an equal amount of water. Each treatment was repeated three times. The plot area was 30 m² (6 × 5 m). After 5, 10, 15, 20, 25, and 30 days, the second leaf was taken to determine the indexes (Section 2.3). The application was a hand-operated knapsack sprayer (300 kPa pressure) (China Delixi Holding Group Co., Ltd., Yueqing, China) with a flat fan nozzle. The nozzle was calibrated to spray 450 L/ha.

Table 1. Penoxsulam spray dosage.

Variety	CK g a.i. ha ⁻¹	0.5X g a.i. ha ⁻¹	1X g a.i. ha ⁻¹	2X g a.i. ha ⁻¹	3X g a.i. ha ⁻¹	4X g a.i. ha ⁻¹
Jingu 21	0	15	30	60	90	120

Pot experiments were carried out in the greenhouses of Shanxi Agricultural University. Jingu 21 was planted in pots (7 × 7 cm), and 5–8 plants were planted in each pot. Different doses of penoxsulam (refer to Table 1) were sprayed at the 3–5 leaf stage, and the different treatments were applied five times. At 5, 10, 15, 20, 25, and 30 days after spraying, the second leaf from the top was taken from each plant to determine various indicators (Section 2.3). The illumination period of the greenhouse was 16:8 h (light/dark), the

temperature was 25:18 °C (light/dark), the illumination intensity was 12,000 xl, and the relative humidity was 70–80%. The pot experiment setting was repeated thrice.

Test equipment: 3WP-2000 Bioassay Spray Tower (developed by Nanjing Institute of Agricultural Mechanization, Ministry of Agriculture and Rural Affairs, Nanjing, China). The spray time could be set at will, and the foxtail millet was placed on the sample plate in the tower. The distance between the sample plate and the nozzle was 250–750 mm, adjustable. After the door of the tower is closed, the environment inside the tower will not be affected by external airflow and other factors, as shown in Figure 1. The walking distance was 1340 mm, and the walking speed of the nozzle was 497 mm/s. The flow rate was 390 mL/min, the effective spray swath was 350 mm, and the spraying time was 2.7 s.



Figure 1. 3WP-2000 bioassay spray tower.

2.3. Determination Indicators and Methods

To assess the response to herbicide stress:

(1) Observation method

The main symptoms of herbicide damage are as follows:

Color changes: macula, yellowing, etc.;

Morphological changes: dwarfing, deformity, leaf curling, withering, etc.

(2) Agronomic traits measuring method

Five plants with uniform growth were selected in each treatment, and the plant height was measured with a ruler. To calculate the leaf area, first determine the length and width of the second leaf of the plant from top to bottom, and then use the following formula [31]:

$$\text{Leaf area} = \text{leaf length} \times \text{leaf width} \times 0.75 \quad (1)$$

(3) Photosynthetic characteristics measuring method

After applying the penoxsulam, five plants with the same growth were randomly selected from each treatment and three leaves were collected. After removing the veins, weigh 0.05 g of fresh samples, slice them, put them in a 10 mL tube, and soak them in

5 mL of 96% anhydrous ethanol for 24 h until the leaves turn white. During the extraction process, shake the tube every 6–8 h to fully mix the photosynthetic pigment with ethanol. At 470, 649, and 665 nm, the absorption rate, chlorophyll a, chlorophyll b, and carotenoid content were measured by UV 2400 spectrophotometer (Shunyu Henping Instrument, LLC, Shanghai, China) and calculated by the following formula [32]:

$$C_a = 13.95 \times A_{665} - 6.88 \times A_{649} \quad (2)$$

$$C_b = 24.96 \times A_{649} - 7.32 \times A_{665} \quad (3)$$

$$C_{car} = 1000 \times A_{470} - 2.05 \times C_a - 114.8 \times C_b / 245 \quad (4)$$

$$\text{Pigment content (mg g}^{-1} \text{ FW)} = C \times V_T \times \frac{n}{FW} \times 1000$$

where C is the pigment concentration (mg L⁻¹), FW is the fresh weight (g), V_T is the total volume of the extraction (mL), and n is the dilution ratio.

Photosynthetic gas exchange parameters, including the net photosynthetic rate (*P_n*), transpiration rate (*Tr*), stomatal conductance (*G_s*), and intercellular CO₂ concentration (*C_i*), were measured using CI-340 (CID Bio-Science, Inc., Washington, USA) in the field experiment and LI-6800 (Li-Cor, Inc., Nebraska, USA) in the pot experiments. Photosynthetic gas exchange parameters were measured from 10:00 to 11:00 a.m. The photosynthetically active radiation at the leaf surface was 900 ± 50 μmol m⁻² s⁻¹, the temperature of the leaf chamber was 30 ± 2 °C, and the ambient CO₂ concentration was 400 ± 50 μmol mol⁻¹. Chlorophyll fluorescence parameters, including the maximum photochemical efficiency (*F_v/F_m*), PSII actual photochemical efficiency Y(II), photochemical quenching coefficient (*qP*) and non-photochemical quenching coefficient (NPQ), were measured using MINI-PAM-II (Walz, Effeltrich, Germany).

(4) Efficacy of herbicide

Ten and thirty days after treatment, the number of weed plants [*Echinochloa crus-galli* (L.) Beauv. (*E. crus-galli*); *Digitaria sanguinalis* (L.) Scop. (*D. sanguinalis*); *Chenopodium album* L. (*C. album*); *Amaranthus retroflexus* L. (*A. retroflexus*)] in each treatment plot and plant weight was investigated by a 5-point sampling method; each point was 0.25 m² (0.5 × 0.5 m) and later converted into the weed number control effect and fresh weight control effect.

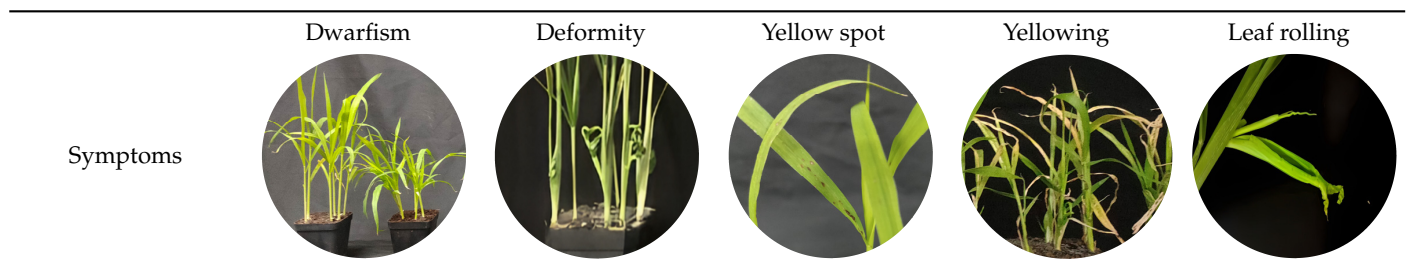
2.4. Data Processing

All experiments were conducted in a completely randomized design with three replications, and the pot plant experiment was conducted three times. Data processing was performed using Microsoft Excel 2021 (Microsoft, Redmond, WA, USA) and IBM SPSS Statistics 25 software (SPSS Inc., Chicago, IL, USA). Duncan's new multiple range method was used for the analysis of variance and multiple comparisons (significance was considered at the *p* < 0.05 level). The statistics were measured by GraphPad Prism 9 (Graphpad software, LLC, San Diego, CA, USA) and Origin 2021 (Originlab, Northampton, MA, USA). Data were presented as mean ± sem.

3. Results

3.1. Observation of Herbicide Damage

The symptoms of penoxsulam's damage to Jingu 21 are shown in Table 2, and these mainly include dwarfism, deformity, macula, yellowing and leaf rolling. These symptoms are more obvious with the increase in spraying dose. The higher the concentration of penoxsulam, the more serious the harm of the herbicide, and the degree of harm is 4X > 3X > 2X > 1X > 0.5X.

Table 2. Damage symptoms of foxtail millet caused by penoxsulam herbicide.

3.2. Effects of Spraying Different Doses of Penoxsulam on Agronomic Traits

3.2.1. Effects of Penoxsulam on Height of Foxtail Millet

In the pot and field experiments, plant height decreased following treatment with penoxsulam, with the decrease in plant height proportional to the spraying dose. In the pot experiment, the height of Jingu 21 increased in the 0.5X and 1X spraying dose groups throughout the assessed timeframe, whereas, plant height in the 2X spraying dose group only increased after 30 days of application. However, plant height in the 3X and 4X spraying dose groups did not increase significantly after 5–30 days (Figure 2).

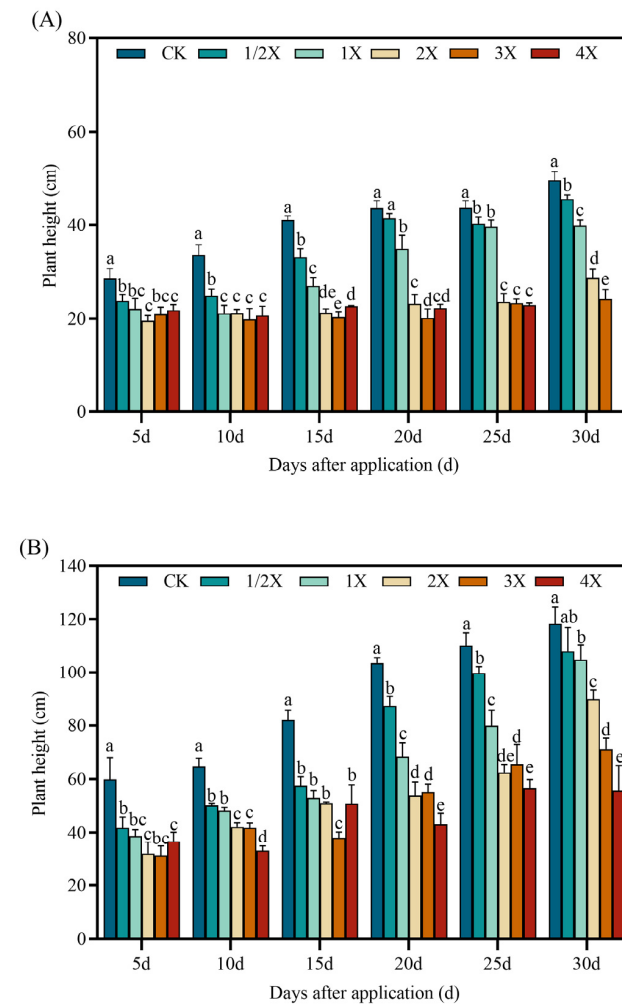


Figure 2. Effect of penoxsulam on plant height of foxtail millet (A) pot experiment, (B) field experiment). Error bars indicate the standard error of means. In the pot plant experiment, 30 days after spraying, all the plants under the 4X treatment of foxtail millet died. Note: Comparison between treatments of different concentrations on the same day, with lowercase letters representing a significant difference ($p < 0.05$).

In the pot experiment, the plant height of foxtail millet sprayed with penoxsulam was significantly reduced by 25.84–40.80% compared with 0 (control, CK) at 10 days after spraying. In the field experiment, the plant height under a 4X spraying dose was significantly reduced by 49.20% 10 days after spraying (Figure 2).

3.2.2. Effects of Penoxsulam on Leaf Area of Foxtail Millet

In the pot and field experiments, the leaf area decreased significantly following treatment with penoxsulam, with the leaf area decreasing as the spraying dose increased (Tables 3 and 4). In the pot experiment, the leaf area of foxtail millet sprayed with penoxsulam was significantly reduced by 38.91–71.49%, compared with CK, after 5 days of spraying. On day 15, in the 2X, 3X, and 4X spraying dose groups, due to phytotoxicity and plant deformity, measurements of the leaf area were difficult to obtain. After 30 days of treatment, the leaf area of the 3X spraying dose group was significantly reduced by 80.56% compared with CK (Table 3).

Table 3. Effects of penoxsulam on the leaf area of Jingu 21 (pot plant experiment). “—” means that the plant was deformed, and the leaf area could not be measured.

Treatment	Leaf Area (cm ²)					
	5 d	10 d	15 d	20 d	25 d	30 d
CK	12.98 ± 1.0 a	17.04 ± 1.0	23.26 ± 0.8 a	25.27 ± 2.2 a	25.31 ± 0.5 a	36.06 ± 1.2 a
0.5X	7.93 ± 1.3 b	10.38 ± 1.4	15.51 ± 0.9 b	21.5 ± 1.6 ab	20.39 ± 0.2 b	31.37 ± 0.8 b
1X	4.83 ± 0.7 c	—	9.39 ± 0.9 c	16.8 ± 1.7 b	20.1 ± 2.0 b	20.42 ± 0.9 c
2X	5.04 ± 0.7 bc	—	—	8.62 ± 1.0 c	6.22 ± 0.9 c	9.77 ± 1.9 d
3X	3.7 ± 0.9 c	—	—	—	6.09 ± 0.8 c	7.01 ± 0.8 d
4X	4.29 ± 1.2 c	—	—	—	—	—

Note: Comparison between treatments of different concentrations on the same day, with lowercase letters representing a significant difference ($p < 0.05$).

Table 4. Effects of penoxsulam on the leaf area of foxtail millet (field experiment). “—” means that the plant was deformed, and the leaf area could not be measured.

Treatment	Leaf Area (cm ²)					
	5 d	10 d	15 d	20 d	25 d	30 d
CK	54.63	74.49	98.60 ± 4.7 a	117.33 ± 5.0 a	112.84 ± 8.1 a	92.22 ± 4.0 a
0.5X	—	—	56.52 ± 7.4 b	86.60 ± 7.6 b	80.87 ± 2.1 b	89.59 ± 5.5 a
1X	—	—	46.23 ± 5.6 b	69.07 ± 5.1 b	72.32 ± 4.2 b	89.55 ± 4.2 a
2X	—	—	—	28.42 ± 1.9 c	56.16 ± 3.1 c	74.86 ± 5.6 b
3X	—	—	—	37.87 ± 6.7 c	49.34 ± 0.4 c	45.18 ± 1.3 c
4X	—	—	—	—	32.74 ± 5.2 d	37.94 ± 4.1 c

Note: Comparison between treatments of different concentrations on the same day, with lowercase letters representing a significant difference ($p < 0.05$).

In the field experiment, on days 5 and 10, the leaf area of each spraying dose group was difficult to measure because of phytotoxicity and plant deformity. After 15 days of treatment, the leaf area of the 0.5X and 1X spraying dose groups was significantly reduced by 42.68%, and 53.11%, respectively, compared with CK.

3.3. Effects of Spraying Different Doses of Penoxsulam on Photosynthetic Characteristics

3.3.1. Effects of Penoxsulam on Photosynthetic Pigment Content of Foxtail Millet

In both the pot experiment and the field experiment, the contents of Chla, Chlb, Car, and total chlorophyll decreased by different degrees in the different spraying dose groups. The overall trend was that the contents of Chla, Chlb, Car, and total chlorophyll decreased gradually with an increase in spraying dose (Figures 3 and 4).

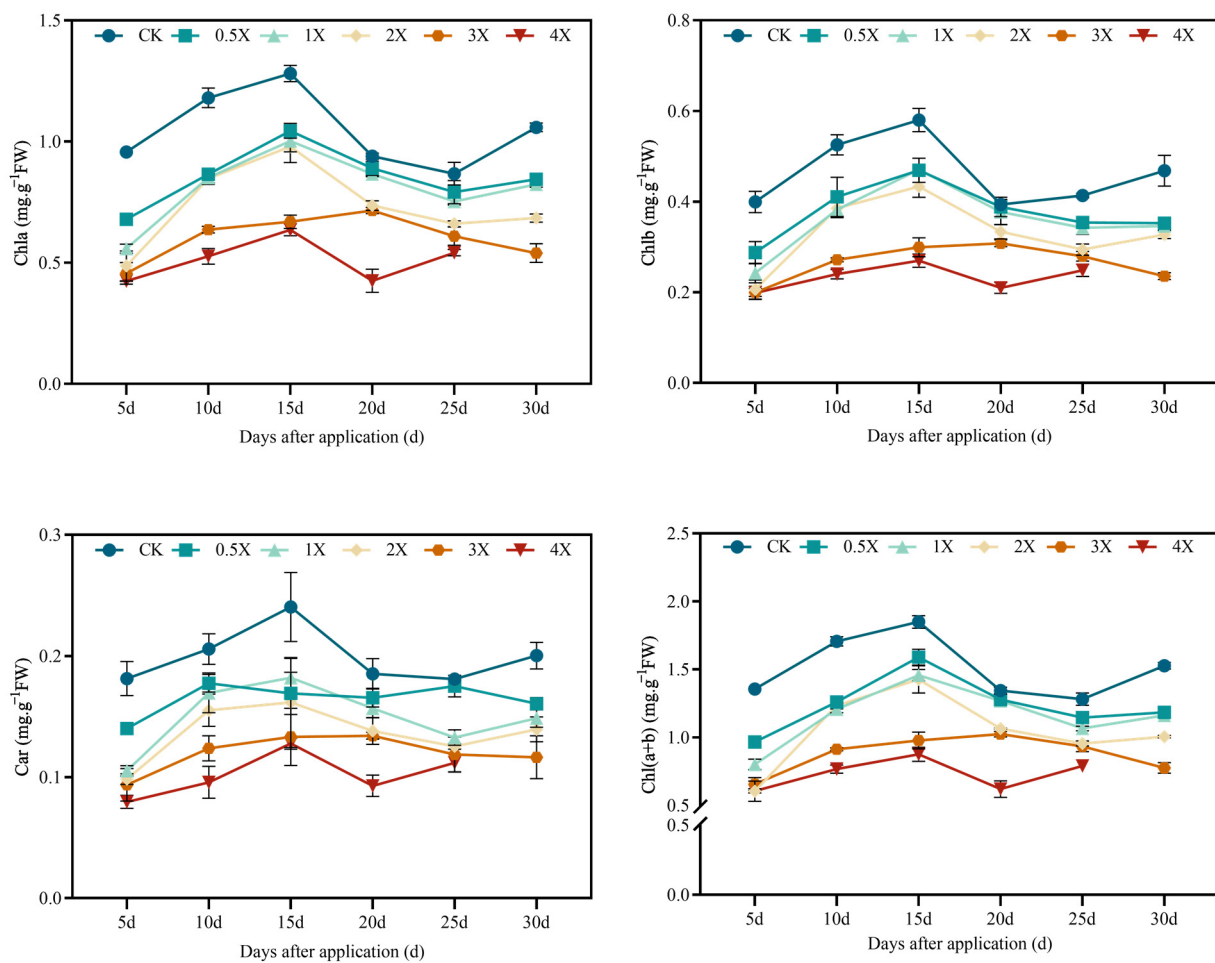


Figure 3. Effects of penoxsulam on photosynthetic pigment content in foxtail millet leaves (pot plant experiment). In the pot plant experiment, 30 days after spraying, all the plants under the 4X treatment of foxtail millet died.

In the pot experiment, the content of Chlb under a 4X spraying dose was significantly reduced by 54.28%, compared with CK, at 10 days after spraying. After 25 days of treatment, the Chla content of the foxtail millet under penoxsulam treatment was significantly reduced by 8.76–37.55%. After 25 days of treatment, the Chla content in each group was significantly reduced by 8.76–37.55% compared with CK. In the field experiment, the Chla content was significantly reduced by 15.90%, 23.62%, 25.62%, and 31.03% compared with CK on day 5, for the 1X, 2X, 3X, and 4X spraying dose groups, respectively (Figures 3 and 4).

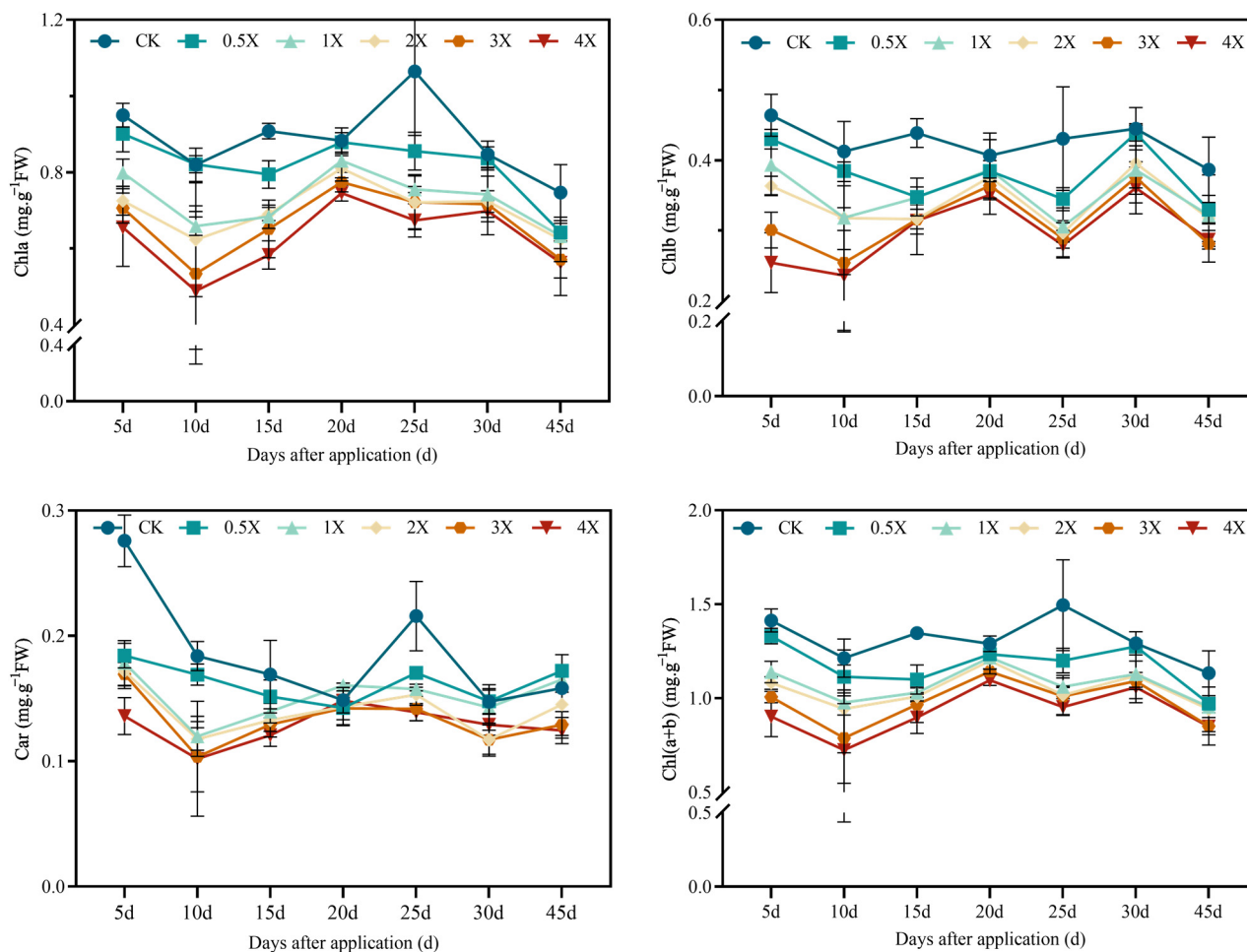


Figure 4. Effects of penoxsulam on photosynthetic pigment content in foxtail millet leaves (field experiment).

3.3.2. Effects of Penoxsulam on Photosynthetic Parameters of Foxtail Millet

In the pot and field experiments, the P_n decreased significantly after penoxsulam treatment, with the P_n decreasing proportionally as the spraying dose increased (Figure 5).

In the pot experiment, the P_n in 4X spraying-dose group was significantly reduced by 86.76% compared with CK on day 5. There was no significant difference between P_n and CK 20–30 days after spraying. In the field experiment, the P_n was significantly reduced by 25.77%, 23.47%, and 31.95% compared with CK in the 2X, 3X, and 4X spraying dose groups after 10 days of treatment. After 30 days of treatment, the P_n under the 4X spraying dose group treatment was significantly reduced by 24.28% compared with CK (Figure 5).

The Tr decreased significantly after application of penoxsulam in both the pot and field experiments. In all groups, the Tr decreased as the spraying dose increased (Figure 6). In the pot experiment, the Tr was significantly reduced by 63.88% and 74.43%, compared with CK, in the 3X and 4X spraying dose groups after 15 days of treatment. At 25 days after treatment, the Tr was significantly different from CK in the 1X, 2X, 3X, and 4X spraying dose groups. In the field experiment, the Tr was significantly reduced by 12.80%, 11.90%, 18.67%, 31.78%, and 35.24% compared with CK on day 15. On day 30, the Tr was significantly reduced by 40.78% compared with CK (Figure 6).

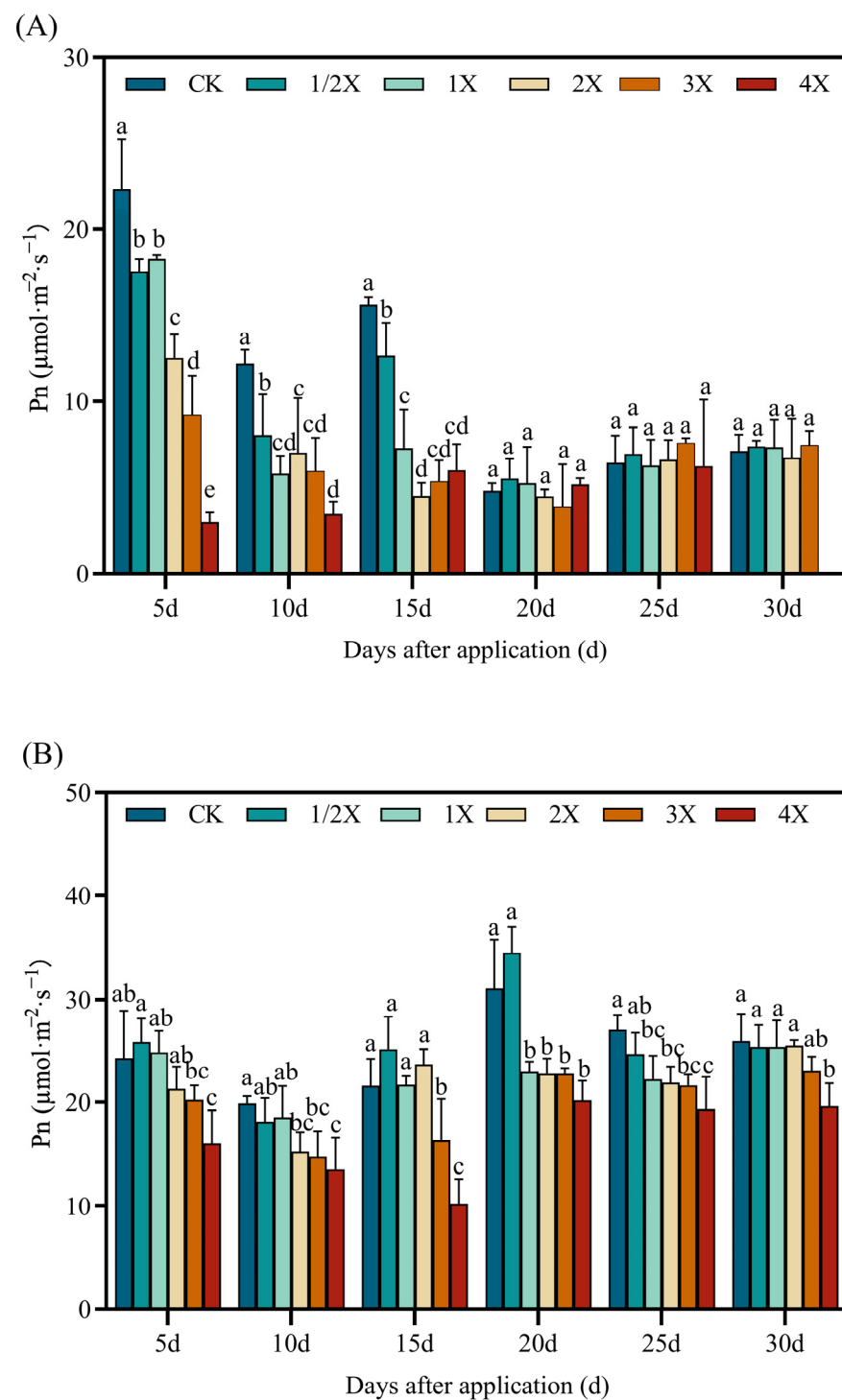


Figure 5. Effects of penoxsulam on P_n in foxtail millet leaves ((A) pot experiment, (B) field experiment). Error bars indicate the standard error of means. In the pot plant experiment, 30 days after spraying, all the plants under the 4X treatment of foxtail millet died. Note: Comparison between treatments of different concentrations on the same day, with lowercase letters representing a significant difference ($p < 0.05$).

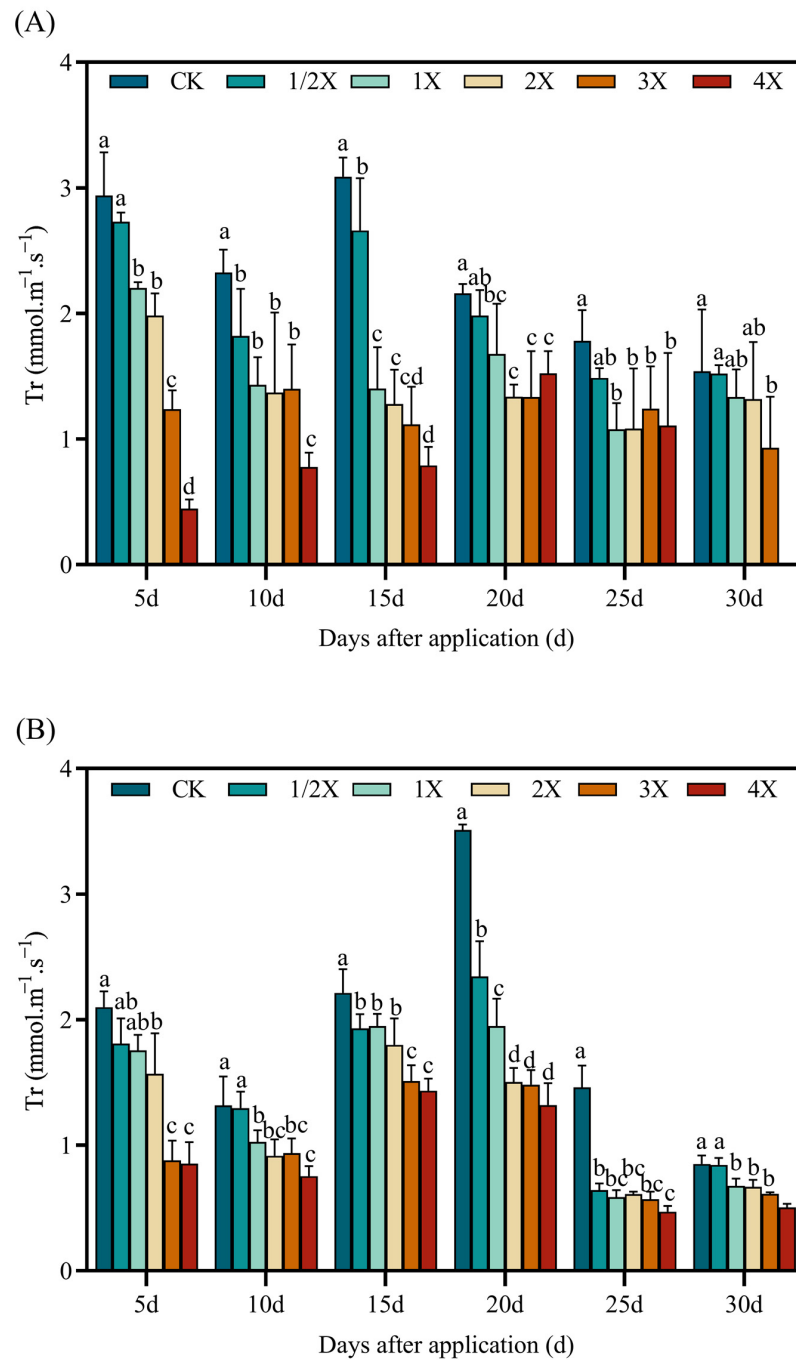


Figure 6. Effects of penoxsulam on *Tr* in foxtail millet leaves ((A) pot experiment, (B) field experiment). Error bars indicate the standard error of means. In the pot plant experiment, 30 days after spraying, all the plants under the 4X treatment of foxtail millet died. Note: Comparison between treatments of different concentrations on the same day, with lower-case letters representing a significant difference ($p < 0.05$).

In the pot experiment, the *Gs* decreased significantly after the treatment, and the overall trend was that the *Gs* decreased with an increase in spraying dose (Figure 7). Also, in the pot experiment, the *Gs* was significantly reduced by 10.53%, 27.80%, 37.69%, 62.36%, and 83.97% compared with CK at 5 days after spraying. After 25 days of treatment, the *Gs* was significantly reduced by 52.58% compared with CK in the 4X spraying dose group. In the field experiment, there was no significant difference among the 0.5X, 1X, 2X, and 3X groups compared to CK after 10 days. After 25 days of treatment, the *Gs* was significantly

reduced by 62.53% compared with CK in the 4X spraying dose group. At 30 days after treatment, there was no significant difference in stomatal conductance among the 2X, 3X, and 4X spraying dose groups, but it was significantly lower in all groups compared to the CK group (Figure 7).

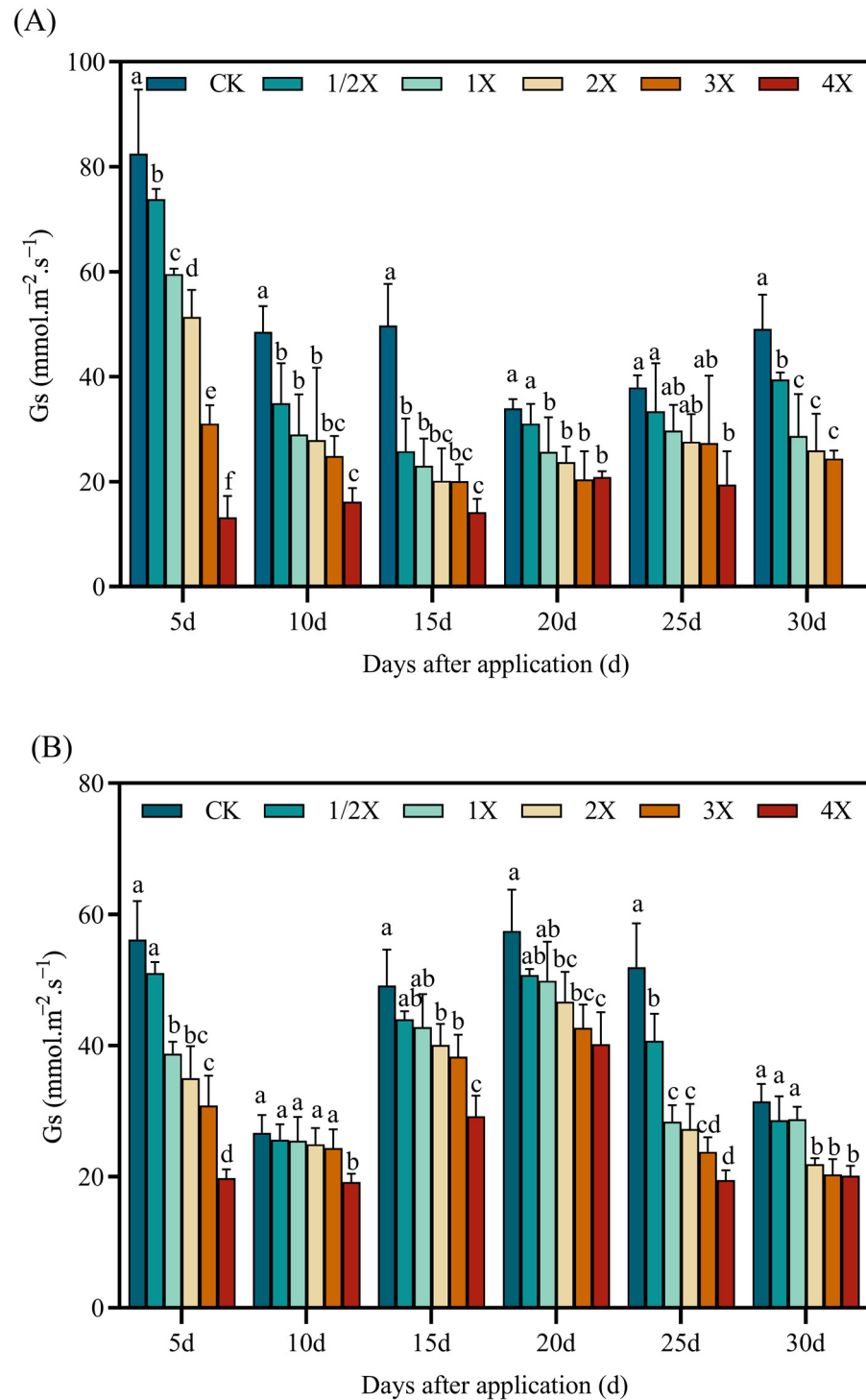


Figure 7. Effects of penoxsulam on Gs in foxtail millet leaves ((A) pot experiment, (B) field experiment). Error bars indicate the standard error of means. In the pot plant experiment, 30 days after spraying, all the plants under the 4X treatment of foxtail millet died. Note: Comparison between treatments of different concentrations on the same day, with lower-case letters representing a significant difference ($p < 0.05$).

In both the pot and field experiments, the C_i increased significantly after treatment with penoxsulam, with the increase in line with the spraying dose increase (Figure 8). In the pot experiment, the C_i was significantly increased by 35.69% compared with CK in the 4X spraying dose group at 10 days after treatment. After 25 days of spraying, the C_i increased significantly by 69.51%, 46.69%, 45.08%, 140.34%, and 201.52% compared with CK. In the field experiment, 25 days after treatment, the C_i of the 4X spraying dose group was significantly increased by 5.52% compared with CK, and there was no significant difference among the 1X, 2X, and 3X spraying dose groups. After 30 days, the C_i in the 4X spraying dose group was significantly higher than that of the other groups (Figure 8).

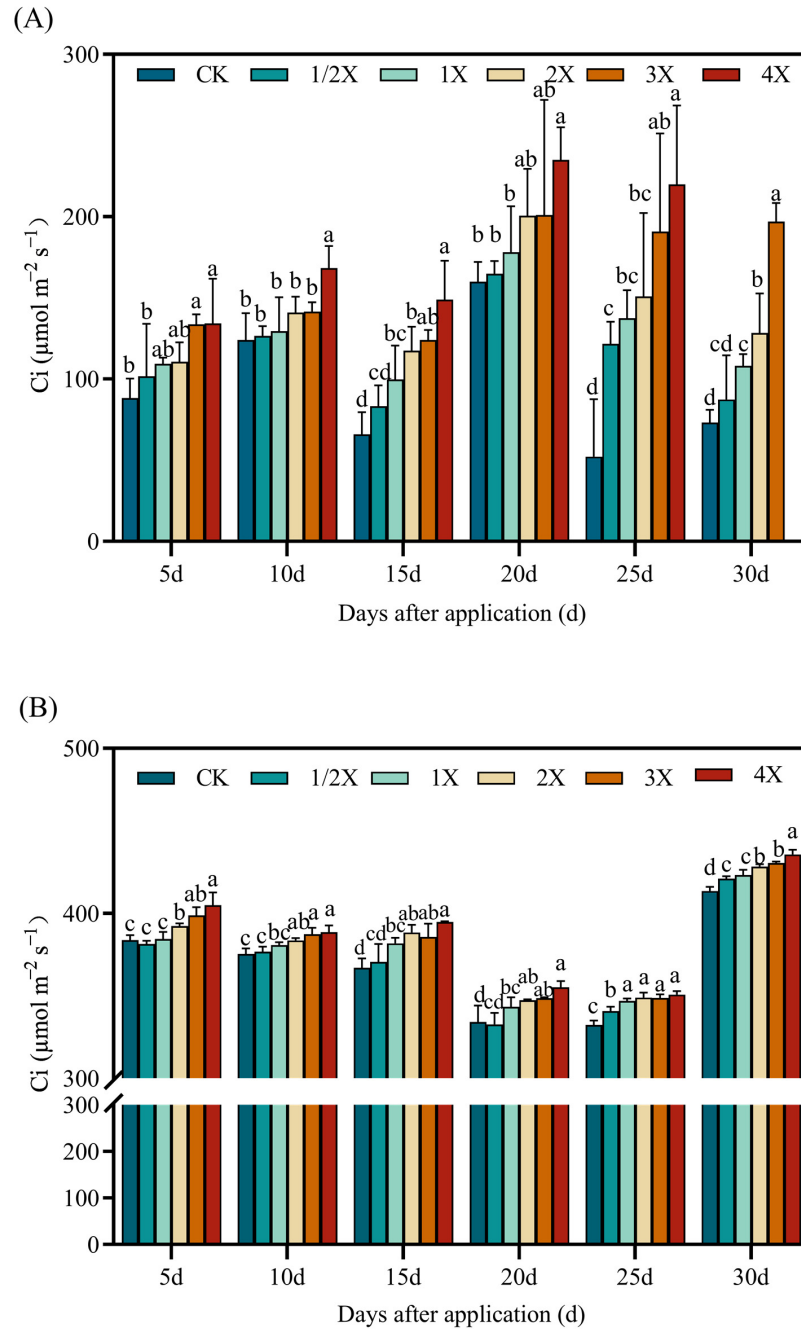


Figure 8. Effects of penoxsulam on C_i in foxtail millet leaves ((A) pot experiment, (B) field experiment). Error bars indicate the standard error of means. In the pot plant experiment, 30 days after spraying, all the plants under the 4X treatment of foxtail millet died. Note: Comparison between treatments of different concentrations on the same day, with lower-case letters representing a significant difference ($p < 0.05$).

3.3.3. Effects of Penoxsulam on Chlorophyll Fluorescence Parameters

In the pot and field experiments, the concentration of penoxsulam affected Fv/Fm , $Y(II)$, and qP . The overall trend was that Fv/Fm , $Y(II)$, and qP decreased as the spraying dose increased. This is in contrast to NPQ, which increased as the spraying dose of penoxsulam increased (Figures 9 and 10).

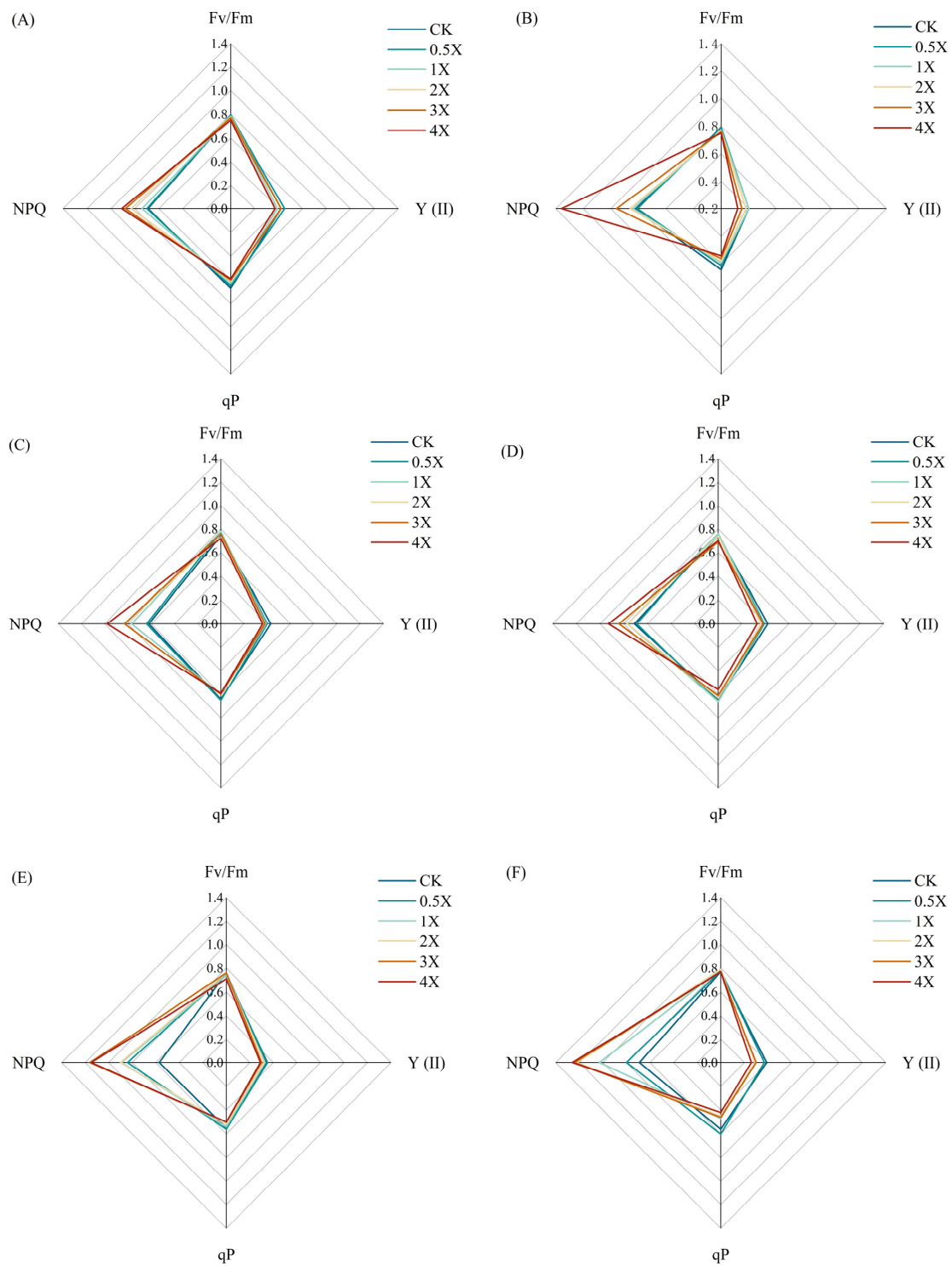


Figure 9. Effects of Penoxsulam on chlorophyll fluorescence parameters in foxtail millet leaves (pot plant experiment). In the pot plant experiment, 30 days after spraying, all the plants under the 4X treatment of foxtail millet died. (A) 5d, (B) 10d, (C) 15d, (D) 20d, (E) 25d, (F) 30d.

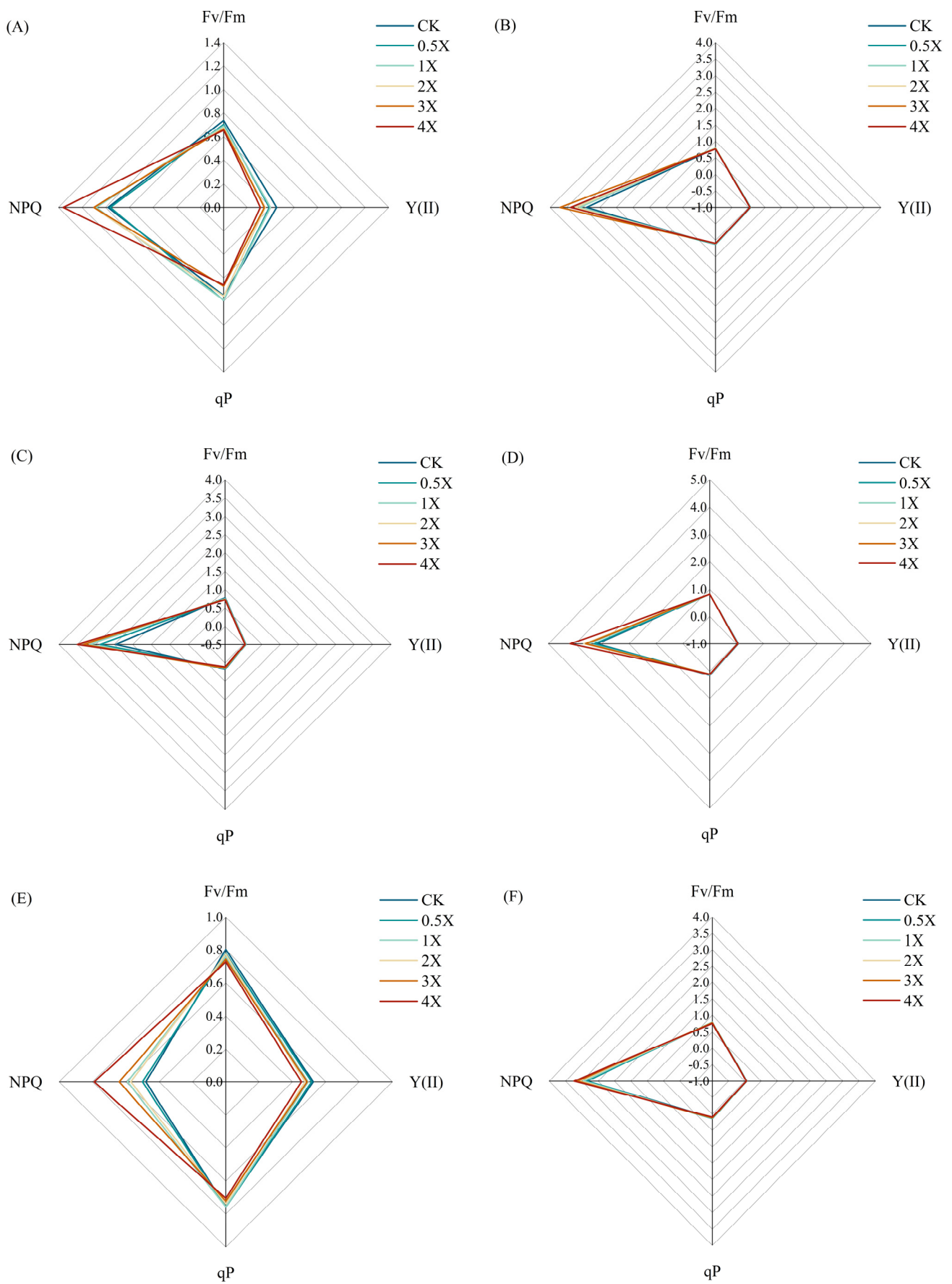


Figure 10. Effects of Penoxsulam on chlorophyll fluorescence parameters in foxtail millet leaves (field experiment). (A) 5d, (B) 10d, (C) 15d, (D) 20d, (E) 25d, (F) 30d.

In the pot experiment, there was no significant difference in $Y(II)$ and qP 5 days after spraying. There was no significant difference in Fv/Fm , $Y(II)$, and qP between CK and the treatment groups on day 15, and there was no significant difference between qP and CK on day 30 (Figure 9). In the field experiment, the Fv/Fm leaves were significantly reduced by 6.11%, 8.33%, 9.47%, and 11.01% in the 1X, 2X, 3X, and 4X spraying dose groups compared with CK on day 5. On day 30, there was no significant difference among all groups in $Y(II)$ and qP , compared with CK (Figure 10).

3.4. Efficacy of Penoxsulam

When penoxsulam was applied for 10–30 days, its ability to control the growth of the weed plants *E. crus-galli*, *D. sanguinalis*, *C. album*, and *A. retroflexus* was apparent, and it was especially effective against *E. crus-galli* and *A. retroflexus*. However, its growth-inhibitory effect against *D. sanguinalis* and *C. album* was poor. The ability of penoxsulam to control weed growth increased as the spraying dosage increased. The growth-inhibiting effect of penoxsulam on *E. crus-galli* and *A. retroflexus* was also spraying time-dependent, with the most pronounced effects recorded following longer application times, with a 100% growth inhibition of *E. crus-galli* recorded at 30 days with 2X, 3X, and 4X spraying doses. At 10 days post-treatment, the control of *A. retroflexus* by penoxsulam was poor, ranging from 5% to 50%. However, after 30 days, 100% control was achieved. In contrast, the control effect on *D. sanguinalis* differed significantly at 30 days post-application among the spraying dose groups. The fresh weight inhibition rate of *E. crus-galli* was 30 d after application, and all spraying doses were higher than 95%. The fresh weight inhibition rate of *E. crus-galli* was 100% at 30 d after application. The fresh weight inhibition rate of *C. album* was higher than 92% in all spray dosage groups on the 30th day after application (Figures 11 and 12).

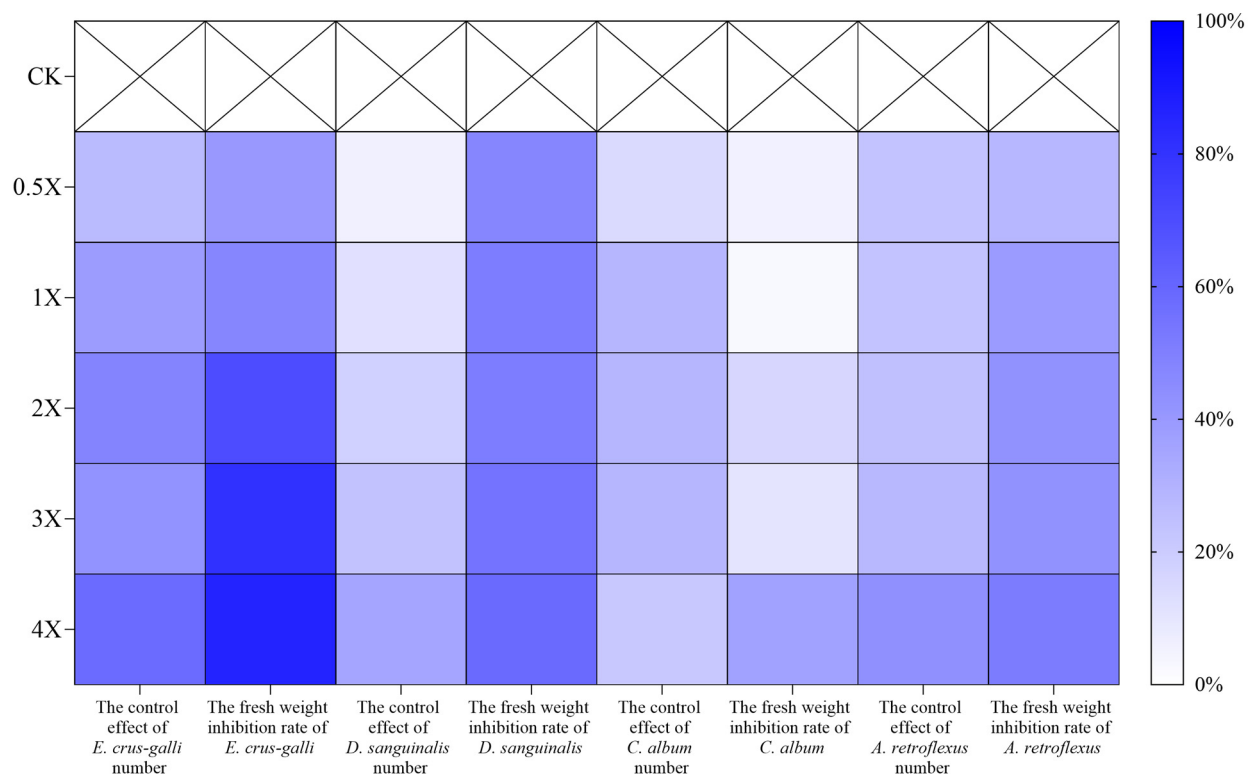


Figure 11. Efficacy of penoxsulam (ten days after application).

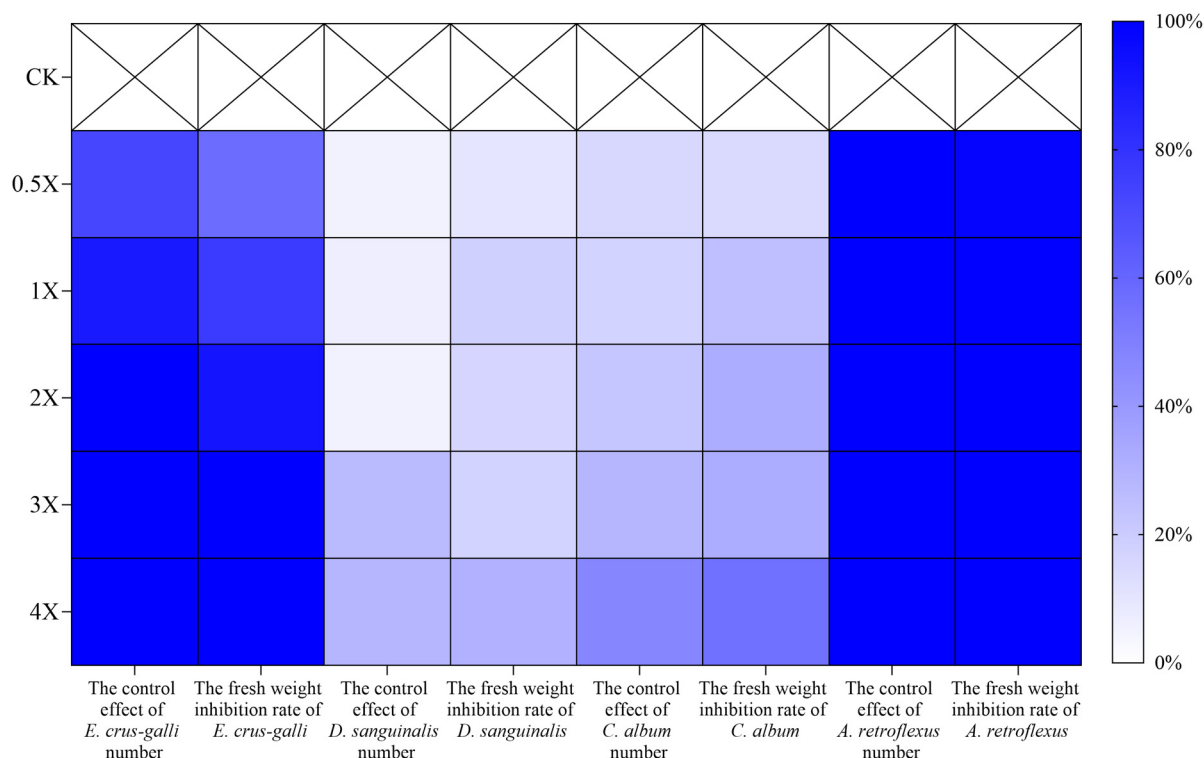


Figure 12. Efficacy of penoxsulam (thirty days after application).

4. Discussion

In this experiment, penoxsulam had a certain control effect on *E. crus-galli*, *D. sanguinalis*, *C. album*, and *A. retroflexus*, which increased with an increase in spraying dose, and all reached the maximum at 4X. However, at this dose, penoxsulam caused serious pesticide damage to the foxtail millet, which was similar to the research results of Zhang et al. [33]. The control effect of herbicides on weeds could be shown as a certain dose–effect relationship.

Agronomic traits such as plant height and leaf area are important methods for measuring crop growth, especially following stress by the application of herbicides. Previous studies have shown that herbicides can adversely affect the growth of corn [34], cotton [35], and soybean [36] crops. In a study of the effects of herbicides on crop growth and yield, herbicide treatment reduced the vine length, number of leaves, total leaf area, and storage root yield of sweet potato [37]. In another study, pyrazosulfuron-methyl inhibited the growth of foxtail millet seedlings, which was measured using plant height and leaf area. Notably, the agronomic traits did not recover significantly in the long-term, indicating that pyrazosulfuron-methyl causes irreversible damage to foxtail millet seedlings [38]. It was also found that, after 30 days of exposure, prometryn seriously inhibits the growth of beans, reducing plant height and leaf area, an indication that stress status had been induced [31]. After different doses of penoxsulam treatment, plant height and leaf area were measured to evaluate the safety of foxtail millet. In this study, through field experiments, it was found that after 1–30 days of application, foxtail millet growth was significantly reduced compared to the control group. Notably, the foxtail millet in the 4X spraying dose group exhibited very little growth over 30 days. Furthermore, plant height decreased proportionally as the penoxsulam spraying dose increased. During the leaf area measurements, it was found in both the pot and field experiments that the leaves were deformed, and the leaves were closed and could not open. With an increase in spraying time, the curled leaves gradually recovered, and the measured leaf area was not significantly different from that of CK.

Different types of herbicides affect photosynthesis in different ways. The impact of herbicide stress on photosynthesis is mainly manifested through it increasing stomatal conductance, reducing pigment synthesis, inhibiting photosynthetic electron transport, and interfering with photosynthetic carbon assimilation [39].

The fluorescence parameters of chlorophyll are important factors that affect plant photosynthesis and electron transfer [40]. They can be used to measure the effects of herbicide stress on crops [41,42]. Yuan et al. [24] showed that chlorsulfuron treatment can increase the Pn and Gs [43]. The current study found that the photosynthetic parameters of Pn , Gs , Tr , Fv/Fm , $Y(II)$, ETR , and qP decreased as the spraying dose of penoxsulam increased. In contrast, the Ci and NPQ content increased in a dose-dependent manner. The increase in Ci indicated that the decrease in Pn was caused by non-stomatal factors. These findings are in line with those previously reported regarding the impact of bensulfuron methyl on the Pn of soybean and peanut, which was postulated to be caused by non-stomatal factors [44]. Fv/Fm represents the light energy conversion efficiency in the PSII reaction center. Generally, Fv/Fm decreases when plants are stressed [45]. Consistent with our results, it was reported that sethoxydim has similar effects on the photosynthetic pigments, photosynthetic gas exchange, and chlorophyll fluorescence parameters of foxtail millet [32].

Under the five spraying doses, the 0.5X spraying dose group was safe for foxtail millet, the degree of phytotoxicity was small, and growth could be quickly restored. The 1X spraying dose group was relatively safe for foxtail millet, the degree of phytotoxicity was small, and growth would gradually recover after 15–20 days after treatment. The 2X spraying dose group had a greater degree of phytotoxicity to Jingu 21, and the recovery growth rate was slow. The 3X and 4X spraying dose groups had a greater degree of phytotoxicity to foxtail millet.

With an increase in spraying dosage, the plant height and leaf area of foxtail millet recovered with time. There was still a significant difference compared with CK, which may be due to the deformity of Jingu 21 after spraying penoxsulam. Penoxsulam may cause tillering, which leads to the distribution of nutrients absorbed by foxtail millet from its roots to different tillers and damaged leaves. Furthermore, it will lead to a lack of nutrients in the stem. This study showed that penoxsulam reduced the photosynthetic rate of foxtail millet, and the non-stomatal factors caused by it may be the main reason for the decrease in photosynthetic rate of the foxtail millet. The PSII complex of foxtail millet leaves was damaged, the openness of the PSII reaction center was reduced, the activity was weakened, the photochemical efficiency was reduced, and the absorbed light energy was more dissipated in the form of heat. The increase in NPQ can be regarded as a self-regulation mechanism of foxtail millet leaves to a herbicide stress environment. This experiment only studied the effects of spraying penoxsulam on the agronomic traits and photosynthetic characteristics of foxtail millet, and its safety evaluation was not comprehensive enough. In the future, it is necessary to continue to study other indicators. At the same time, it is necessary to continue to study the effect of penoxsulam on other foxtail millet varieties.

5. Conclusions

Penoxsulam had a growth-inhibiting effect on *E. crus-galli*, *D. sanguinalis*, *C. album*, and *A. retroflexus*, and it increased with the increase in spraying dosage, reaching the maximum at 4X; but at this dosage, penoxsulam was seriously harmful to foxtail millet. In penoxsulam, within 15 days after spraying, the growth of foxtail millet was slow, the plant was deformed, the plant height and leaf area decreased with the increase in spraying dosage; the plant height and leaf area gradually recovered 15–25 days after spraying, but there were still significant differences compared with the control. The photosynthetic pigment content, Pn , Tr , Gs , Fv/Fm , $Y(II)$, and qP of Jingu 21 all showed a downward trend with the increase in the penoxsulam spraying dose, while the Ci and NPQ increased with the time after spraying. The 0.5X and 1X spraying dose groups were safe for foxtail millet and could be used to control gramineous weeds in fields. However, due to the high toxicity

of other spraying dose groups to plants, they are not recommended for use in the field. In the future, penoxsulam and its possible combinations with other herbicides should be studied to control weeds, reduce toxicity, and delay the development of herbicide resistance in foxtail millet fields.

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