Photosynthetic Physiological Response of Radix Isatidis (Isatis indigotica Fort.) Seedlings to Nicosulfuron

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Abstract

Radix Isatidis (Isatis indigotica Fort.) is one of the most important traditional Chinese medicine plants. However, there is no suitable herbicide used for weed control in Radix Isatidis field during postemergence stage. To explore the safety of sulfonylurea herbicide nicosulfuron on Radix Isatidis (Isatis indigotica Fort.) seedlings and the photosynthetic physiological response of the plant to the herbicide, biological mass, leaf area, photosynthetic pigment content, photosynthetic rate, chlorophyll fluorescence characteristics, and $P_{\text{700}}$ parameters of Radix Isatidis seedlings were analyzed 10 d after nicosulfuron treatment at 5th leaf stage in this greenhouse research. The results showed that biological mass, total chlorophyll, chlorophyll $a$, and carotenoids content, photosynthetic rate, stomatal conductance, PS II maximum quantum yield, $PS\text{ II} \text{ effective quantum yield, PS } II \text{ electron transport rate, photochemical quenching, maximal } P_{700} \text{ change, photochemical quantum yield of PS I, and PS I electron transport rate decreased with increasing herbicide concentrations, whereas initial fluorescence, quantum yield of non-regulated energy dissipation in PS II and quantum yield of non-photochemical energy dissipation due to acceptor side limitation in PS I increased. It suggests that nicosulfuron } \geq 1 \text{ mg L}^{-1} \text{ causes the damage of chloroplast, PS II and PS I structure. Electron transport limitations in PS I receptor side, and blocked dark reaction process may be the main cause of the significantly inhibited growth and decreased photosynthetic rate of Radix Isatidis seedlings.}

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Introduction

As one of the most important traditional Chinese medicine plants, Radix Isatidis (Isatis indigotica Fort.), one kind of cruciferous plants, is extensively cultivated in China. However, weeds seriously limit its yield and quality. Compared with conventional manual control of weeds, chemical control is more effective, yet there is no suitable herbicide used for weed control in Radix Isatidis field during postemergence stage [1]. Nicosulfuron (2-((4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl)-N, N-di-methylnicotinamide) belongs to the acetyl lactic acid synthase (ALS) inhibitor, and can effectively control many perennial and annual grasses as well as certain broadleaf weeds [2,3]. Is nicosulfuron safe to Radix Isatidis seedlings or not?

It is reported that nicosulfuron can effectively control large crabgrass (Digitaria sanguinalis) cornfield, goosegrass (Eleusine indica), spiny amaranth (Amaranthus spinosus L), amaranthus blitum (Amaranthus ascendenis Loisel.), speargrass (Imperata cylindrica), and purslane (Portulaca oleracea) in maize field [4]. However, there are significant differences during the sensitivity of maize varieties to nicosulfuron [5], and sweet corn is much more sensitive to nicosulfuron than the others [6]. The leaves of sensitive maize variety may show symptoms of chlorosis and shrinking 7 d after applying nicosulfuron [7]. The weeding efficiency of nicosulfuron herbicide is related to weed species, leaf age and how long it is applied after rain. [8]. Meanwhile, the resistance of plant to nicosulfuron depends on its metabolic rate, sensitivity to ALS, and nicosulfuron dosage [9–11].

However, there is few paper reporting the application of post-emergence herbicide controlling many grasses and broadleaf weeds in Radix Isatidis fields [1]. It is shown that rational application of trifluralin, pendimethalin and glyphosate at pre-emergence stage can control weeds effectively in Radix Isatidis fields [12,13]. It is also reported that clethodim and quizalofop-p-ethyl at post-emergence stage can be used for grassy weed control in Radix Isatidis fields [12,14].

Primary mode of action of the ALS-inhibiting herbicides that interfere with the activity of ALS enzyme seems no longer in doubt [15]. But, secondary effects of ALS inhibition, such as decreased photosynthesis, disturbed respiration, and synthesis of branched chain amino acids, etc., need to be investigated, which have also been implicated in the mechanism of plant death. Detection of
chlorophyll fluorescence dynamics is a rapid and non-invasive probe of researching plant photosynthetic functions, which has been widely applied to study the effects of herbicides in plants [1,16–19]. Therefore, the objectives of this research were to (1) assess the possibility of application of nicosulfuron in Radix Isatidis field, and (2) understand the related photosynthetic physiological mechanism.

Materials and Methods

Materials and experiment design

Radix Isatidis (Isatis indigotica Fort.) seeds were supplied by Anguo Lixin Medicinal Materials Co., Ltd., Hebei province, China. Nicosulfuron (40%, OF) was provided by Xianda Chemical Co., Ltd., Shandong province, China.

This study was conducted in a greenhouse at Shanxi Agricultural University, China. The experiment was designed as a randomized complete block design with three replications and each replicate containing three pots. Fifteen Radix Isatidis seeds were grown equidistantly in 23-cm diam containers filled with a 1:2 mixture of sand and loam soil with 57.2 g kg⁻¹ of organic matter, 0.92 g kg⁻¹ of total nitrogen, 14.37 mg kg⁻¹ of available phosphorus, and 114.3 mg kg⁻¹ of rapidly-available potassium. The seeds were covered with 1 cm of 1:2 sand/soil mixtures and each pot was carefully watered. Seedlings were grown under greenhouse conditions of 24±3°C day/night temperatures and were thinned to three plants per container at three-leaf stage.

Radix Isatidis seedlings were treated at five-leaf stage. These plants were treated with 0, 0.5, 1, 2, and 4 times the labeled use concentrations of nicosulfuron in corn. The herbicide product contained 40% nicosulfuron, large amounts of water and a small amount of oil adjuvants, and the recommended effective concentrations were 1 mg L⁻¹ nicosulfuron. Herbicides were applied with a laboratory pot-sprayer equipped with a nozzle, calibrated to deliver 450 L ha⁻¹. Agronomic characters and photosynthetic physiology parameters of Radix Isatidis seedling were determined every 10 d after herbicide treatment. Except the control, seedlings treated with nicosulfuron wilted or died 20 d after herbicide treatment, so the data 10 d after herbicide treatment was determined.

Measurements

The third fully expanded leaf of Radix Isatidis seedlings were sampled for the following tests. Leaf area was determined using a laser leaf area meter CI-203 and the CI-203CA conveyor attachment (United States CID Inc.).

Photosynthetic gas exchange was analyzed with a GFS-3000 optical instrument (Germany WALZ company) which can control photosynthesis by means of light intensity, leaf temperature, air flow rate and CO₂ concentration in the cuvette. Photosynthetic rate (P₆₅₀), transpiration rate (Tᵥ), stomatal conductance (Gᵥ) and intercellular CO₂ concentrations (Cᵥ) were measured simultaneously with the light intensity at (800±0.4 μmol m⁻² s⁻¹) and CO₂ concentration (379±0.4 μmol mol⁻¹). Air flow rate was set at 750 μmol s⁻¹ and air temperature (20.9±0.4°C) was also recorded automatically by the instrument. Stomatal limitation value (Lᵥ) = 1–Cᵥ/CA (Cᵥ is the atmospheric CO₂ concentration). Non-stomatal limitation value was calculated as Cᵥ/Gᵥ [20]. For the measurement of photosynthetic pigments, leaves were extracted from leaf discs with 80% (v/v) acetone and assayed spectrophotometrically using extinction coefficients according to Porra et al. [21].

Chlorophyll fluorescence and P₇₀₀ parameters were measured simultaneously by Dual-PAM-100 measurement system (Germany WALZ company), using the automated “Induction Curve” routine provided by the Dual PAM software [22]. Prior to measurements, treated plants were placed in darkroom for 30 min, and fluorescence induced curve (Slow Kinetics) was determined in “Flu+P₇₀₀ model”. Then, the kinetics of chlorophyll fluorescence induction and P₇₀₀ oxidation were recorded simultaneously by the instrument. Firstly, the initial fluorescence (Fₒₒ) was established and subsequently the maximum fluorescence (Fₚₚ) was determined by the “Saturation Pulse” method. Secondly, the maximal P₇₀₀ change (P₇₀₀) was determined by application of a saturation pulse (SP) after far-red pre-illumination. Thirdly, actinic illumination was started and SP was given every 20 s, with the same pulses serving for fluorescence and P₇₀₀ analysis.

PS II maximum quantum yield (Fₚₚ/Fₙ₉₉) was evaluated as Fₚₚ/Fₙ₉₉. Other PS II energy dissipation parameters were estimated by the Dual PAM software. qP = (Fₙ₉₉–F)/Fₙ₉₉ was used as indicator to reflect a ratio of light energy absorbed in PS II being used to photochemical electron transport. Apparent electron transfer efficiency in PS II in light was calculated according to ETR(II) = PAR×0.84×0.5×Y(II), and was used to measure electron transfer of carbon fixation resulted from photochemical reactions. Three complementary quantum yields of energy conversion in PS II were calculated: PS II effective quantum yield (Y(II)) was calculated as Y(II) = (Fₚₚ–F)/Fₚₚ; the yield of non-photochemical losses via non-regulated pathways of PS II as Y(NO) = 1/(NPQ+1+qP(Fₖ₉₉/Fₙ₉₉–1)); NPQ = Fₙ₉₉–Fₖ₉₉/Fₙ₉₉, where quantum yield of regulated energy dissipation in PS II as Y(NPQ) = 1–Y(II)–Y(NO) [23].

P₇₀₀ oxidation was monitored by absorbance changes in the near-infrared (830–875 nm) [24]. The maximal P₇₀₀ signal observed upon full oxidation was denoted by Pm. Y(NA), the quantum yield of non-photochemical energy dissipation due to acceptor-side limitation, was calculated according to: Y(NA) = (P₆₅₀–Pₙ₆₅₀)/P₆₅₀. Photochemical quantum yield of PSI as Y(I) was estimated according to Y(I) = (P₆₅₀–P)/P₆₅₀. Quantum yield of non-photochemical energy dissipation due to donor side limitation in PS I as Y(ND) was calculated by Y(ND) = (P–P₆₅₀)/P. The total value of three quantum yields was one: Y(II)+Y(NA)+Y(ND) = 1. The electron transfer efficiency of PS I as ETR(I) was provided by the Dual PAM software.

Statistical analysis

Microsoft Office Excel 2003 and Statistics Analysis System 8.0 were used in statistical analysis of the data. Mean values were compared by a one-way analysis of variance (ANOVA), and Duncan’s test was used to determine the significant differences among the treatments. We used P=0.05 as the statistical significance threshold.

Results

Effect of nicosulfuron on agronomic characteristics of Radix Isatidis seedlings

The effects of nicosulfuron on agronomic characteristics of Radix Isatidis seedlings are shown in Table 1. Fresh weight of Radix Isatidis seedlings decreased with the increasing concentrations of nicosulfuron. Leaf area was significantly decreased by nicosulfuron at 4 mg L⁻¹, whereas this was not affected by other treatment. The whole plant fresh weight and shoot fresh weight showed the similar trend, and differences between the treatment of nicosulfuron at 0.5 mg L⁻¹ and the control were significant. Shoot fresh weight declined by 41.64%, 52.90%, 53.58% and 59.04%, respectively, from 0.5 to 4 mg L⁻¹ of nicosulfuron. The reduction in root fresh weight was not significant by nicosulfuron, suggesting
that the suppression of nicosulfuron on the aboveground parts of Radix Isatidis seedlings was greater than root.

**Effect of nicosulfuron on photosynthetic pigment contents in leaves of Radix Isatidis seedlings**

As shown in Table 2, each nicosulfuron treatment caused different degrees of decline in photosynthetic pigment contents in leaves of Radix Isatidis seedlings. It seems that 1 mg L$^{-1}$ of nicosulfuron inhibited $Chl$ by 33.57%, $Chl$ a by 37.04% and $Car$ by 47.37%, and the differences between the treatment and the control were significant. However, $Chl$ b was not significantly decreased until the herbicide concentrations reached up to 2 mg L$^{-1}$, and it was inhibited by 37.14%. Although nicosulfuron declined chlorophyll a/b, there was no significant effect.

**Effect of nicosulfuron on photosynthetic characteristics in leaves of Radix Isatidis seedlings**

Nicosulfuron decreased $P_n$ and $G_i$ in leaves of Radix Isatidis seedling significantly. As concentration of nicosulfuron increases, inhibition of $P_n$ changed by 58.21%, 70.02%, 70.69%, and 78.89%, respectively (Table 3). $G_i$ inhibition also increases as concentration of the herbicide increases by 86.03%, 91.10%, 91.21%, and 96.49%, respectively when nicosulfuron varies from 0.5 to 4 mg L$^{-1}$ (Table 3). However, the results of $P_n$ and $G_i$ revealed no significant differences ($P>0.05$) from 1 to 4 mg L$^{-1}$ of nicosulfuron. $G_i$ increased first then declined with increasing of nicosulfuron concentrations, peaked at 0.5 mg L$^{-1}$, and was the lowest at 4 mg L$^{-1}$. Compared to $G_i$, the change trend of $L_n$ was just the opposite. $L_n$ was the least at 4 mg L$^{-1}$, and the largest at 1 mg L$^{-1}$. For $G_i/G_n$, the value for treated plants was higher than the control in different degrees.

**Effect of nicosulfuron on chlorophyll fluorescence parameters in leaves of Radix Isatidis seedlings**

As shown in Fig 1-A, $F_o$ increased with the increase in nicosulfuron concentration. $F_o$ was a bit higher than the control for 2 mg L$^{-1}$ treatment, but significantly higher for 4 mg L$^{-1}$ treatment. $F_o/F_m$ was slightly higher than the control for 0.5 mg L$^{-1}$ concentration, and then decreased with the increase concentration of nicosulfuron.

Changes of $Y(II)$, $ETR$ and $q_P$ were consistent, and declined in a concentration-dependent manner. Compared with the control, $Y(II)$ was reduced by 7.01%, 36.07%, 70.57% and 81.95%, $ETR$ declined by 7.10%, 36.14%, 70.44% and 81.91%, and $q_P$ decreased by 8.64%, 29.54%, 70.19% and 82.51%, respectively (Fig 1-B, 1-D).

$Y(NO)$ showed a fluctuant increase as nicosulfuron concentration increases, slightly higher than the control for 0.5 mg L$^{-1}$ and 1 mg L$^{-1}$ concentration, and significantly (49.70%) higher for 2 mg L$^{-1}$. Compared to $Y(NO)$, the trend of $Y(NPQ)$ was the opposite (Fig 1-C). It showed that the degree of the relative excess light damage induced by nicosulfuron in Radix Isatidis leaf was more severe with increasing nicosulfuron concentration.

**Effect of nicosulfuron on $P_{700}$ parameters in leaves of Radix Isatidis seedlings**

Increasing nicosulfuron concentration induced a decline in $P_{700}$, while the differences between 1 mg L$^{-1}$ and 2 mg L$^{-1}$ were not significant (Fig 2-A). As shown in Fig 2-B, the trend of $ETR$ (I) and PS I was consistent, and the values of them at 2 mg L$^{-1}$ were much smaller than 1 mg L$^{-1}$ (Fig 2-B). In Fig 2-C, nicosulfuron induced an increase of $Y(NA)$, while $Y(ND)$ increased first and then decreased with the increasing of nicosulfuron concentration, and the peak was at 1 mg L$^{-1}$ (Fig 2-C).

### Table 1. Effect of different concentrations of nicosulfuron on agronomic traits of radix isatidis seedlings.

| Nicosulfuron (mg L$^{-1}$) | LA (cm$^2$)   | WFW (g)   | SW (g)   | RFW (g)   |
|---------------------------|---------------|-----------|----------|-----------|
| 0                         | 10.54±0.24$^a$| 3.18±0.04$^a$| 2.93±0.05$^a$| 0.25±0.05$^a$|
| 0.5                       | 9.93±0.75$^a$ | 1.94±0.18$^b$| 1.71±0.16$^b$| 0.23±0.03$^b$|
| 1                         | 11.11±0.37$^a$| 1.58±0.10$^{bc}$| 1.38±0.04$^{bc}$| 0.20±0.04$^{bc}$|
| 2                         | 9.54±0.24$^a$ | 1.52±0.14$^{bc}$| 1.36±0.22$^{bc}$| 0.17±0.02$^{bc}$|
| 4                         | 7.37±0.71$^b$ | 1.36±0.29$^{bc}$| 1.20±0.18$^{bc}$| 0.16±0.01$^{bc}$|

Note: The data in the table is mean ± SD. The different letters in the same column indicate significantly different at $P<0.05$ level by Duncan’s new multiple range test. LA, WFW, SW, and RFW represent leaf area, the whole plant fresh weight, shoot fresh weight, and root fresh weight, respectively.

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| Nicosulfuron (mg L$^{-1}$) | $Chl$ (mg g$^{-1}$) | $Chl$ a (mg g$^{-1}$) | $Chl$ b (mg g$^{-1}$) | $Car$ (mg g$^{-1}$) | $Chl$ a/b |
|---------------------------|---------------------|-----------------------|-----------------------|---------------------|-----------|
| 0                         | 1.43±0.10$^a$       | 1.08±0.08$^a$         | 0.33±0.01$^a$         | 0.19±0.01$^a$       | 3.12±0.11$^a$|
| 0.5                       | 1.00±0.03$^b$       | 0.75±0.02$^b$         | 0.25±0.05$^b$         | 0.13±0.02$^b$       | 2.98±0.68$^b$|
| 1                         | 0.95±0.05$^c$       | 0.68±0.01$^c$         | 0.27±0.04$^c$         | 0.10±0.01$^c$       | 2.49±0.35$^c$|
| 2                         | 0.82±0.07$^d$       | 0.60±0.09$^d$         | 0.22±0.02$^d$         | 0.12±0.01$^d$       | 2.76±0.61$^d$|
| 4                         | 0.57±0.11$^d$       | 0.40±0.09$^d$         | 0.17±0.02$^d$         | 0.08±0.02$^d$       | 2.34±0.30$^d$|

Note: The data in the table is mean ± SD. The different letters in the same column indicate significantly different at $P<0.05$ level by Duncan’s new multiple range test. $Chl$, $Chl$ a, $Chl$ b, $Car$, and $Chl$ a/b represent chlorophyll, chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a/b, respectively.

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The safety of herbicides on plants may be represented through agronomic traits and physiological indexes [1,25,26]. In this study, nicosulfuron at the recommended usage (1 mg L\(^{-1}\)) was not safe to Radix Isatidis seedlings, which was reflected by reduced biomass. It was coincident with Yuan et al. [1], mesosulfuron-iodosulfuron was unsafe to Radix Isatidis seedlings and decreased the leaf area.

| Nicosulfuron (mg L\(^{-1}\)) | \(P_n\) (\(\mu\)mol CO\(_2\) m\(^{-2}\) s\(^{-1}\)) | \(C_i\) (\(\mu\)mol mol\(^{-1}\)) | \(G_s\) (mmol H\(_2\)O m\(^{-2}\) s\(^{-1}\)) | \(L_s\) | \(C_i/G_s\) |
|-----------------------------|-----------------|-----------------|-----------------|-------|-------|
| 0                           | 11.94±1.857\(^b\) | 296.30±10.62\(^a\) | 264.57±76.31\(^a\) | 0.21±0.03\(^a\) | 1.16±0.29\(^a\) |
| 0.5                         | 4.99±0.23\(^b\)  | 336.31±13.18\(^c\) | 36.96±0.24\(^a\)  | 0.11±0.04\(^a\) | 9.10±0.30\(^a\)  |
| 1                           | 3.58±1.26\(^b\)  | 309.20±70.20\(^d\) | 23.54±13.04\(^c\) | 0.05±0.11\(^b\) | 19.98±7.05\(^d\) |
| 2                           | 3.50±0.60\(^b\)  | 235.73±63.10\(^d\) | 23.25±14.89\(^c\) | 0.38±0.17\(^a\) | 13.85±1.58\(^d\) |
| 4                           | 2.52±1.57\(^c\)  | 115.10±10.38\(^c\) | 9.29±17.92\(^b\)  | 0.70±0.01\(^b\) | 21.60±2.32\(^a\) |

Note: The data in the table is mean±SD. The different letters in the same column indicate significantly different at \(P<0.05\) level by Duncan’s new multiple range test. \(P_n\), \(C_i\), \(G_s\), \(L_s\) and \(C_i/G_s\) represent photosynthetic rate, intercellular CO\(_2\) concentrations, stomatal conductance, stomatal limitation value, and non-stomatal limitation value, respectively.

**Discussion**

The safety of herbicides on plants may be represented through agronomic traits and physiological indexes [1,25,26]. In this study, nicosulfuron at the recommended usage (1 mg L\(^{-1}\)) was not safe to Radix Isatidis seedlings, which was reflected by reduced biomass. It was coincident with Yuan et al. [1], mesosulfuron-iodosulfuron was unsafe to Radix Isatidis seedlings and decreased the leaf area.

![Figure 1: Effect of nicosulfuron on chlorophyll fluorescence parameters in leaves of Radix Isatidis seedlings.](https://www.plosone.org/article/fichiers/10.1371/journal.pone.0105310.g001)

Values represent the means and vertical bars indicate the standard deviation of three separate experiments. \(F_o\), \(F_m\), \(Y(\text{II})\), \(ETR(\text{II})\), \(Y(\text{NO})\), \(Y(\text{NPQ})\) and \(q_P\) represent initial fluorescence, PS II maximum quantum yield, PS II electron transport rate, PS II effective quantum yield, quantum yield of non-regulated energy dissipation in PS II, quantum yield of regulated energy dissipation in PS II, and photochemical quenching, respectively. The abscissa in the figure represents the concentration of nicosulfuron and the unit is “mg L\(^{-1}\).”

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and fresh weight significantly. Meanwhile, nicosulfuron was unsafe to Radix Isatidis seedlings being reflected by reduced chlorophyll content and $P_n$.

Is the decline in $P_n$ resulted by stomatal factors or non-stomatal factors? In our current research, $P_n$ and $G_s$ at 0.5 mg L$^{-1}$ were significant lower than the control, however, $C_i$ and $C_i/G_s$ were a little higher than the control. It may suggest that the decline in $P_n$ is mainly caused by stomatal limitation. $P_n$, $G_s$ and $C_i$ treated by nicosulfuron at 2 mg L$^{-1}$ were significantly lower than the control, and $L_s$ and $C_i/G_s$ were significant higher than the control, showing that both stomatal and non-stomatal factors may limit the photosynthesis. Nicosulfuron at 0.5 mg L$^{-1}$ decreased Chl a and Car content significantly. It may suggest that nicosulfuron destructs the chloroplast structure of Radix Isatidis leaf, reduces the thylakoid stacking level [27], increases the risk of photo-oxidation damage, reduces the light absorption, transmission, distribution between PS II and PSI [28], and affects the synthesis of ATP and NADPH.

Chloroplasts of photosynthetic apparatus, PS II and PS I in thylakoid membranes are the most sensitive parts to environmental changes. Reversible inactivation or damage of PS II reaction center can cause increase in $F_o$ [29]. Paraquat and norflurazon at 100 mg L$^{-1}$ significantly reduced $F_v/F_m$, $Y(II)$ and $q_P$ in leaves of Lemma minor, but enhanced NPQ markedly [30]. Acetochlor and fluoroglycofen decreased the photochemical efficiency of photosystem II ($Y(II)$) in the light and increased non-photochemical quenching (NPQ) [17]. Previous studies [19] have shown that Sigma Broad causes damage to PS II complex, block photosynthetic electron transfer, reduce $F_v/F_m$, $Y(II)$ and $q_P$ significantly, and lead to increase in initial fluorescence, quantum yield of non-regulated energy dissipation in PS II in Radix Isatidis seedlings. Similar to the previous, $F_o$ and $Y(NO)$ increased, whereas $F_v/F_m$, $Y(II)$, ETR(II), $q_P$ and $Y(NPQ)$ decreased in leaves treated by nicosulfuron in this study. It may suggest that nicosulfuron causes excess excitation energy accumulation in PS II reaction center, the higher reduction state of $Q_A$, net loss of D1 protein, reversible deactivation or destruction in PS II reaction centers [31], opening percentage to decrease, harmful effect on photosynthetic oxygen-evolving complex, electron transport efficiency to decline, ATP and NADPH to reduce in Radix Isatidis leaves.

Figure 2. Effect of nicosulfuron on P700 parameters in leaves of Radix Isatidis seedlings. Values represent the means and vertical bars indicate the standard deviation of three separate experiments. $P_{m}$, $Y(I)$, ETR (I), $Y(ND)$ and $Y(NA)$ represent maximal $P_{m}$ change, photochemical quantum yield of PS I, PS I electron transport rate, quantum yield of non-photochemical energy dissipation due to donor side limitation in PS I, and quantum yield of non-photochemical energy dissipation due to acceptor side limitation in PS I. The abscissa in the figure represents the concentration of nicosulfuron and the unit is “mg L$^{-1}$”.
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research, \( P_{\text{net}} \), \( Y(\text{T}) \) and \( ETR(\text{T}) \) declined with the increase of nicosulfuron usage, and which at 1 mg L\(^{-1}\) were significantly lower than the control. It may suggest that herbicide suppressed the activity of PS I in Radix Isatidis leaves, and electron transfer was blocked at its receptor side. \( Y(\text{ND}) \) reflects the state of electron donor in PS II, and it is affected by the transmembrane proton gradient and PS II damage degree. \( Y(\text{NA}) \) reflects the state of electron acceptor in PS I, and it is affected by dark adaptation and \( \text{CO}_2 \) fixation damage level. \( Y(\text{NA}) > 0.5 \) mg L\(^{-1}\) increased significantly, showing that nicosulfuron aggravated the injury of PS II in Radix Isatidis leaves, declined transmembrane proton gradient, blocked dark reaction process, and reduced the fixed amount of \( \text{CO}_2 \).

In conclusion, recommended usage of nicosulfuron for maize is not safe to Radix Isatidis seedlings. It causes the damage of chloroplast, PS II and PS I structure. Electron transport limitations in PS I receptor side, blocked dark reaction process may be the main cause of the significantly inhibited growth and decreased photosynthetic rate of Radix Isatidis seedlings. Effect of nicosulfuron on the activities of key enzymes in the Calvin cycle of Radix Isatidis seedlings will be researched in the later experiment.

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Author Contributions

Conceived and designed the experiments: XY PG LZ. Performed the experiments: XY LZ NN MY. Analyzed the data: XY LZ SD. Contributed reagents/materials/analysis tools: BW LF YW MG. Wrote the paper: XY LZ.

References

1. Yuan X, Guo P, Qiu X, Ning N, Wang H, et al. (2013) Safety of herbicide Sigma Broad on Radix Isatidis (Isatis indigotica Fort.) seedlings and their photosynthetic physiological responses. Pestic Biochem Physiol 106: 45–50.

2. Lum AF, Chikoye D, Adesiyan SO (2005) Control of Imperata cylindrica (L.) Raenichel (sparegras) with nicosulfuron and its effects on the growth, grain yield and essential components of maize. Crop Prot 24 (4): 41–47.

3. Hennigh DS, Al-Khatib K, Taintora MR (2010) Response of Acetolactate synthase-resistant grain sorghum to nicosulfuron plus rimsulfuron. Weed Technol 24 (4): 411–415.

4. Zhang L, Hu Y, Wang W, Guo L, Wang Y (2012) Controlling effect of nicosulfuron 6% SC on weeds in corn fields. Weed Sci 30 (2): 58–60. (in Chinese with English abstract).

5. Dong X, Wang J, Bi J, Liu Y, Zhang X (2007) The sensitivity of different maize varieties to the nicosulfuron. Acta Phytologia Sinica 34 (2): 182–196. (in Chinese with English abstract).

6. Jonathan NN, Williams MM (2008) A common genetic basis in sweet corn inbred lines for resistance to acetochlor and fluoroglycofen. Pestic Biochem Physiol 103: 210–218.

7. Moro FFV, Damiao Filho CF (1999) Morphological and anatomical alterations in the photosynthetic apparatus of Setaria virdis (Poaceae) and corn. Weed Sci 1: 8–12.

8. Wu J, Mathiassen S, Kudsk P (2003) The influence of weed species, leaf stage and rainfastness on the performance of nicosulfuron. Chin J Pestic Sci 5 (1): 77–81. (in Chinese with English abstract).

9. Sullivan JO, Bouw WJ (1990) Sensitivity of processing sweet corn (Zea mays) cultivars to nicosulfuron/rimsulfuron. Can J Plant Sci 70 (1): 151–154.

10. Geißkler K, Mueller TC, Hayes RM, Schwartz O, Barrett M (1999) Absorption, translocation, and metabolism of primisulfuron and nicosulfuron in broadleaf signalgras (Brachiaria platyphylla) and corn. Weed Sci 1: 8–12.

11. Hemmigh DS, Al-Khatib K (2010) Response of Barnyardgrass (Echinochloa crus-galli), Green Foxtail (Setaria viridis), Longpaine Sandbur (Cenchrus longispinus), and Large Crabgrass (Digitaria sanguinalis) to Nicosulfuron and Rimsulfuron. Weed Sci 3: 189–194.

12. Yuan S, Guo F, Guo Q, Zhang Y, Wang X (2005) Demonstration on chemical weed control in Isatis tectorum L. field. J Shanxi Agric Sci 31: 78–80. (in Chinese with English abstract).

13. Li J, Lu P, Zou Y (2011) The chemical control of Indigowoad root field broad leaves, Value Eng 30: 328. (in Chinese with English abstract).

14. Wang X, Rong R, Zhang J, Yuan X, Wu Y, et al. (2012) Effect of quazadolop on protective enzymes and photosynthesis in Radix Isatidis. J Med Plants Res 6: 1770–1776.

15. Zhou Q, Liu W, Zhang Y, Liu K (2007) Action mechanisms of acetolactate synthase-inhibiting herbicides. Pestic Biochem Physiol 89: 89–96.

16. Kocurek V, Smutny V, Pálova J (2009) Chlorophyll fluorescence as an instrument for the assessment of herbicide efficacy. Cereal Res Commun 37: 299–302.

17. Tan W, Li Q, Zhai H (2012) Photosynthesis and growth responses of grapevine to acetochlor and fluroxypyr. Pestic Biochem Physiol 103: 210–218.

18. Mafridi H, Laurence M, Bruno B, Claude A, Sylvie R, et al. (2006) An easy and rapid method using microscopy to determine herbicide effects in Poaceae weed species. Pest Manag Sci 62: 515–521.

19. Wang Z, Zhou L, Guo W, Zhu X, Li C, et al. (2011) Effects of herbicides on photosynthesis and chlorophyll fluorescence parameters in wheat leaves. J Agro-Environ Sci 30: 1037–1043. (in Chinese with English abstract).

20. Ramanujla S, Sreenivasulu N, Sudhakar C (1998) Effect of water stress on photosynthesis in two mulberry genotypes with different drought tolerance. Photosynthetica 35: 279–283.

21. Fujita H, Kuwahara K, Yasuda T, Sugiyama O (1999) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochem Biophys Acta 197: 304–304.

22. Piendl E, Klaghammer C, Schreiber U (2008) Monitoring the effects of reduced PS II antenna size on quantum yields of photosystems I and II using the Dual-PAM-100 measuring system. PAM Application Notes 1: 21–24.

23. Kramer DM, Johnson G, Kirats O, Edwards GE (2004) New fluorescence parameters for the determination of QA redox state and excitation energy fluxes. Photosynth Res 79: 209–218.

24. Klaghammer C, Schreiber U (2008) Saturation Pulse method for assessment of energy conversion in PS I. PAM Application Notes 1: 11–14.

25. Zhao R, Guo P, Yuan X, Wang J, Han M (2010) Effect of paraquat on the antioxidative enzyme activities and lipid peroxidation in poppy (Papaver somniferum L.). J Plant Dis Protect 2: 55–59.

26. Qian H, Lu T, Peng X, Han X, Fu Z, et al. (2011) Enantioselective phytotoxicity of the herbicide Imaizamox to the response of the antioxidiant system and starch metabolism in Arabidopsis thaliana. PLoS ONE 6 (3): e19451. doi:10.1371/journal.pone.0019451.

27. Liu Z, Shi L, Bai L, Zhao K (2007) Effects of salt stress on the contents of chlorophyll and organic solutes in Aeluropus littoralis var. sinensis Debeaux. J Plant Physiol Mol Biol 33: 165–172.

28. Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007) Photosynthesis of monocotyledonous plants. Photosynthetica 35: 279–283.

29. Scheller HV, Haldrup C (2005) Photoinhibition of photosystem I. Planta 221: 5–9.

30. Wang Z, Zhou L, Guo W, Zhu X, Li C, et al. (2011) Effects of herbicides on photosynthesis and chlorophyll fluorescence parameters in wheat leaves. J Agro-Environ Sci 30: 1037–1043. (in Chinese with English abstract).

31. Schrader SM, Wise RR, Waholtz WF, Ort DR, Sharkey TD (2004) Thylakoid electron transport limitations in PS I receptor side, blocked dark reaction process may be the main cause of the significantly inhibited growth and decreased photosynthetic rate of Radix Isatidis seedlings. Effect of nicosulfuron on the activities of key enzymes in the Calvin cycle of Radix Isatidis seedlings will be researched in the later experiment.