Phytotoxicity Response of Lucern to Herbicide Atrazine in Soil

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ABSTRAT

Limited attention has been given to the persistent impacts of diverse herbicides present in soil on the growth of successive crops in agricultural production. Therefore, the objective of this experiment is to thoroughly examine atrazine residues toxic reactions in lucern (*Medicago sativa L.*). This experiment aims to thoroughly investigate the toxic response of atrazine in lucern. Lucern sourced from Henan Seed Company in China. The study employed the soil addition method to investigate the impacts and correlations of diverse concentrations of atrazine herbicide residues with growth indicators, photosynthetic features, chlorophyll fluorescence parameters of lucern. The results showed that with the increase of atrazine residue (0.0-2.0 mg·kg⁻¹), the plant height (PH), root length (RL), stem dry weight (SDW) and root dry weight (RDW) decreased to 81.8%, 81.7%, 92.3% and 85.2%, respectively. SPAD value, net photosynthetic rate (Pn), stomatal conductance (GS), transpiration rate (Tr), the PSII maximum quantum yield (Fv/Fo), maximum photochemical efficiency (Fv/Fm), actual photosynthetic efficiency (Y(II)), PSII coefficient of photochemical fluorescence quenching (qP) and photosynthetic electron transport rate (ETR) decrease by 62.1%, 83.4%, 84.1%, 95.7%, 76.8%, 11.8%, 84.5%, 46.1% and 63.1%, respectively. However, the intercellular carbon dioxide concentration (Ci) and non-photochemical quenching coefficient (NPQ) increased by 46.2% and 37.5%, respectively. Ci was positively correlated with Fv/Fo, Fv/Fm, qP, Y(II) and ETR (P<0.01), SPAD, Pn and Gs were significantly negatively correlated with Tr (P<0.01), were significantly positively correlated with Tr, Fv/Fo, Fv/Fm, qP, Y(II) and ETR (P<0.01). The potential toxicity risk of atrazine residues to plants was assessed by photosynthetic characteristics and chlorophyll fluorescence parameters. Although herbicide application is essential for food production, appropriate concentration management methods must be adopted to ensure the sustainable development of agricultural ecology.

Keywords: residue, toxic reactions, growth indicators, photosynthetic features, chlorophyll fluorescence parameters, correlation.

INTRODUCTION

The advancement of modern agricultural high-tech heavily depends on the extensive application of chemicals, offering numerous conveniences in crop managing fields (Smedbol et al., 2017). Nevertheless, the infiltration of herbicides into the soil and rivers poses a threat, leading to contamination of subsequent crops and the ecological environment, thereby resulting in economic losses at the national level (Defarge et al., 2016; Kniss, 2017). Atrazine is a common triazine herbicide since the 1980s, with China emerging as the predominant producer and consumer of this chemical. China has consistently maintained a substantial annual consumption of atrazine, with usage reaching tens of thousands of tons. This enduring prominence has been highlighted (Jun Zhang et al., 2014; Stradtman and Freeman, 2021). Lucern has the advantages of clean weeding and affordable price, atrazine finds extensive application in the management of broadleaf weeds, grass weeds, and specific perennial weeds like corn, sugarcane, and sorghum,

as indicated by studies conducted (Sánchez et al., 2017; He et al., 2019; Rostami et al., 2021). Atrazine exhibits a prolonged half-time in the environment, estimated to range between 4 and 57 weeks. Notably, its residual capacity extends well beyond its degradation capabilities. Consequently, atrazine poses a significant risk of inducing toxicity in succeeding crops and negatively impacting the ecological environment (Rong Tan et al., 2015; Bhatt et al., 2022). The extent of plant development works pivotally in determining productivity levels. Residual atrazine in soil can easily cause damage to the development of rice, wheat, and soybeans, leading to a decrease in productivity (Chen et al., 2019; Zhang et al., 2021). Atrazine serves as a typical photosynthetic system II inhibitor. Mainly absorbed through plant roots and transported upward, it affects the photosynthetic process of plants, thereby producing excessive ROS, causing oxidative damage and poisoning non-target plants (Baxter et al., 2016). For instance, millet exhibited significant reductions in biomass and chlorophyll content after receiving atrazine (Sher et al., 2021). Atrazine has the capacity to impede the electron transport chain between electron acceptor protein and plastoquinone by competing with plastoquinone for binding site within photosynthetic center on the thylakoid membrane. This specific interference targets photosystem II (PSII), diminishing CO_2 fixation efficiency, to inhibit their growth rate and photosynthetic efficiency, and cause decrease in plant yield (Bai et al., 2015). Photosynthesis is the transformative process through which plants convert radiant energy into stable chemical energy (Ambavaram et al., 2014). This intricate mechanism encompasses fundamental physiological processes, including the absorption of light energy by plants and the initiation of photochemical reactions. Chlorophyll fluorescence yields valuable insights into the intricacies of photosynthesis, offering a means to figure plants photosynthetic mechanism, subsequently determine the toxicity of residues towards plants. Lucern is one of the perennial leguminous grasses, integral in corn-lucern rotation. In such agricultural practices, there is a propensity for atrazine residues to accumulate in the soil, subsequently impacting the quality of dairy and livestock products while concurrently diminishing alfalfa productivity (Zhang et al., 2014). At present, in the new stage of development of the agricultural sector, there are few reports on the phytotoxicity of atrazine on lucern in corn and alfalfa rotations. This study seeks to replicate and analyze the effects of atrazine soil residue on the growth, photosynthetic characteristics and chlorophyll fluorescence parameters of lucern. It is evident that soil residues have a detrimental effect on the developmental progress of subsequent crops, diminishing their production potential and posing a threat to the safety of the ecological environment.

MATERIALS AND METHODS

Plant culture conditions

Appropriate quantity of lucern seeds was selected based on seed viability and uniformity, and 5% sodium hypochlorite solution was utilized for surface disinfection, subsequent sent to a thorough rinsing with sterile distilled water after a 15-minute exposure. Subsequently, we evenly distributed the seeds on wet gauze within a petri dish, and germinated the seeds in a 25°C incubator until they achieved a white color, then set them aside. The atrazine used in this study had a purity of 99.5% and was sourced from Shanghai Bide Pesticide Factory, China. Soil samples collection was from the topsoil (1–10 cm) in Henan, China, and naturally dried for use (Table. 1) In our experiment, we employed the soil addition method to prepare medicinal soil, with final atrazine concentrations prepared as 0.1, 0.3, 0.5, 1.0, and 2.0 mg·kg⁻¹, with clear water serving as the control. Five germinated lucern seeds were sowed in pots, each treatment was replicated in three pots. These pots were then transferred to controlled conditions within a climate chamber (day: 30 °C, night: 25 °C, relative humidity:70–75%, light intensity: 150 μmol (photon))/(m²·s)).

Growth parameters

The lucern plants were collected 30 days post-sowing. Following this, measurements were taken for both PH and RL of the lucern.

Table 1. Analysis of soil physical-chemical properties

pH	Soil/water ratio	Organic matter (mg·kg⁻¹)	N (mg·kg⁻¹)	P (mg·kg⁻¹)	K (mg·kg⁻¹)
7.0	1:25	5.5	29.5	6.1	78.3

Subsequently, the harvested samples were partitioned into bags, fixed at 105°C for 30 minutes, dried at 80°C for a duration of 48 hours, and subsequently weighed.

Relative chlorophyll content

The chlorophyll content of lucern leaves was measured using a portable chlorophyll meter (SPAD-502Plus).

Gas-exchange measurements

Under optimal environment conditions (CO_2 concentration: 400 µmol/mol, leaf chamber temperature: 28±0.5 °C, light intensity: 150 µmol (photon)/(m²·s)), the LI-6400 portable photosynthetic measurement system was used to assess Pn, Gs, Tr and Ci between 9:00 and 11:00 am on a sunny day. Each experimental treatment was replicated four times.

Chlorophyll fluorescence parameters

The leaves designated for testing were covered with tin foil to induce darkness. Following one hour of dark adaptation, various measurements including Fv/Fm, Fv/Fo, Y(II), ETR, qP and NPQ were taken.

Statistical analysis

Experiment utilized software applications, including Microsoft Excel 2019, DPS v7.05, and GraphPad Prism 9.0, for the purpose of calculation, statistical analysis, and data visualization. All data are depicted as mean ± SD. DPS v7.05 was employed for One-Way ANOVA analysis, with Duncan's new complex range method applied to compare significant indicators identified during variance analysis.

RESULTS AND DISCUSSION

In the current phase of agricultural development, the quality of plant development plays a pivotal role in determining its production potential. Hence, there is a critical need to investigate the influence of soil residues on subsequent crops. For instance, research has shown that bensulfuron-methyl exhibits toxic effects on the growth, development, and chlorophyll fluorescence parameters of cucumber (Sun et al., 2019). Additionally, bensulfuron-methyl exerts severe inhibitory effects on soybeans and peanuts (Su et al., 2018), ultimately resulting in diminished levels of plant development. In this study, the application of atrazine notably impeded the growth of lucern (Fig. 1a). As the concentration of atrazine increased, both lucern PH and RL exhibited substantial decreases. By the 30th day post-treatment, significant differences in PH and RL were observed compared to the control group, particularly with the application of a low concentration (0.1 mg·kg⁻¹). PH witnessed a decline of 15.3%, while RL experienced a reduction of 16.7%. Notably, under high-concentration treatment (2.0 mg·kg⁻¹), both PH and RL of lucern exhibited significant deviations from the control. The reductions were profound, with PH and RL plummeting by 81.8% and 81.7%, respectively, attaining statistical significance. Concurrently, an increase in atrazine residue corresponded to a decrease in dry weight (Fig. 1B). Relative to the control, SDW exhibited reductions ranging from 23.1–92.3%, while RDW decreased by 17.5–85.0%. The primary impact of atrazine residue on lucern manifested as a reduction in biomass. This phenomenon could be attributed to the atrazine residue surpassing lucern tolerance threshold for phytotoxicity, consequently diminishing its production potential (de Sousa et al., 2014). These experimental findings align with earlier research (Wang et al., 2015). Leaf SPAD value demonstrates a positive correlation with chlorophyll content. With an escalation in atrazine concentration, there is a gradual decrease in the SPAD value (Fig. 2). Under the low concentration atrazine treatment (0.1 mg·kg⁻¹), the SPAD value of alucern leaves significantly declined compared to the control, marking a reduction of 10.6%. Conversely, under high concentration (2.0 mg·kg⁻¹) treatment, the SPAD value witnessed a substantial decrease of 62.1%, attaining a highly significant level. The decline in chlorophyll content may be attributed to the herbicide's interference with chlorophyll biosynthesis or the acceleration of chlorophyll decomposition.

Soil herbicide residues potentially impede plant photosynthetic pigments, as well as net photosynthetic rate. For instance, glyphosate has been shown to notably reduce chlorophyll content and Gs in soybeans (Krenchinski et al., 2017). This investigation revealed a gradual decrease in Pn, Gs, and Tr of lucern leaves as the atrazine content in the soil increased (Fig. 3), while Ci gradually increases. At 0.1 mg·kg⁻¹, the lowest concentration,
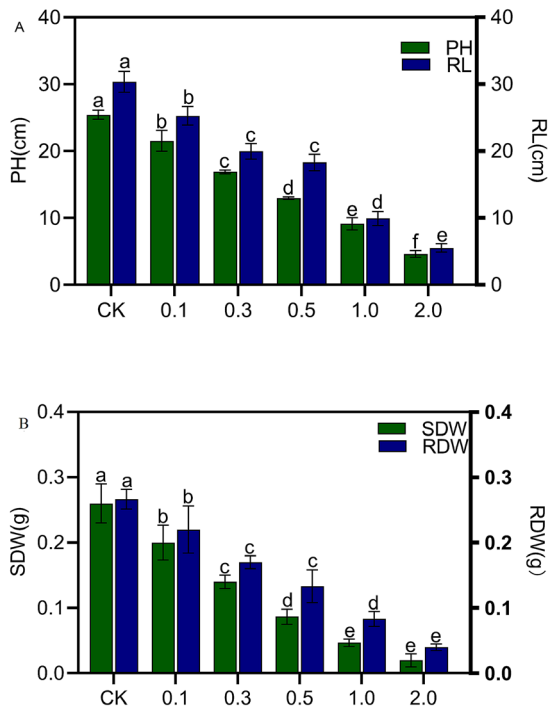
Figure 1. (a) PH and RL, (b) SDW and RDW. Data are means ± SD of three. Duncan was used to analyze the mean values of each parameter, and the significant difference between treatments marked with different lowercase letters was at P < 0.05.
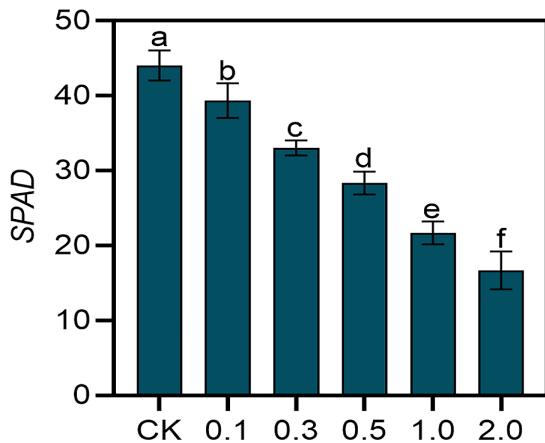
Figure 2. Correlation between atrazine residue distribution and alfalfa leave SPAD value. Duncan was used to analyze the mean values of each parameter, and the significant difference between treatments marked with different lowercase letters was at P < 0.05.

Pn, Gs, and Tr experienced significant reductions of 14.0%, 14.0%, and 27.5%, while Ci exhibited a significant increase of 21.2%. Conversely, at a high dosage (2.0 mg·kg^{-1}), the changes in Pn, Gs, Tr, and Ci were 83.4%, 84.2%, 91.6%, and

46.7%, respectively. These findings indicate a decline in SPAD values with increasing atrazine concentration. A clear dose-effect relationship is evident in the study. The reduction in chlorophyll content may stem from the herbicide's impact on inhibiting chlorophyll biosynthesis or accelerating chlorophyll decomposition. Atrazine residue exerts a suppressive effect on Pn in lucern, with Pn exhibiting similarity to the change in chlorophyll content. The experimental results indicate consistent trends between the two, suggesting that a decrease in Pn may precede a decline in chlorophyll content, marking the initial stage of hindering photosynthesis (Su et al., 2017). Decline of net photosynthetic rate can be due to two distinct factors. One type was stomata-related, leading to a decline in Pn, Gs and Ci. The other type is caused by non-stomatal factors, resulting in reductions in Pn and Gs and upregulation in Ci. Among these, Gs indicates stomatal closure. The parameter is crucial for preventing water loss and CO_2 penetration. Ci signifies the culmination of various driving forces and resistances encountered by external CO_2 gas entering the mesophyll cells, ultimately affecting leaf photosynthesis and respiration balance (Todorova et al., 2022). In this experiment, under atrazine stress, Pn and Gs of lucern decreased, while Ci increased. These results indicate a reduced ability of mesophyll cells to fix CO_2. Non-stomatal closure may be the primary cause of the decrease in Pn. Simultaneously, the decline in photosynthetic performance is associated with electron capture, transfer, and dissipation of PSII. These findings are consistent with earlier research, indicating that Pn inhibition by fumisulfuron is primarily due to non-stomatal closure (Wang et al., 2018).

Chlorophyll fluorescence parameters serve as effective photosynthetic indicators for capture capacity and electron transfer rate. and electron transfer rate and determine the degree of toxicity of herbicides to plants (Hassannejad et al., 2020). Our experimental results showed that atrazine treatment significantly reduced Fv/Fm, Fv/Fo, qP, and ETR of alfalfa, while elevating NPQ. Beyond a concentration of 0.1 mg·kg^{-1}, Y(II), Fv/Fo, ETR, Fv/Fm, and qP of lucern exhibited notable distinctions from control. At 0.3 mg·kg^{-1}, Fv/Fm, Fv/Fo, Y(II), ETR and qP decreased by 4.7%, 30.8%, 30.9%, 24.9% and 20.6% respectively, while NPQ notably differed from the control, increasing by 16.3% at 2.0 mg·kg^{-1} concentration, Fv/Fm, Fv/Fo, Y(II), ETR and qP decreased by 11.8%, 76.9%, 80.0%,
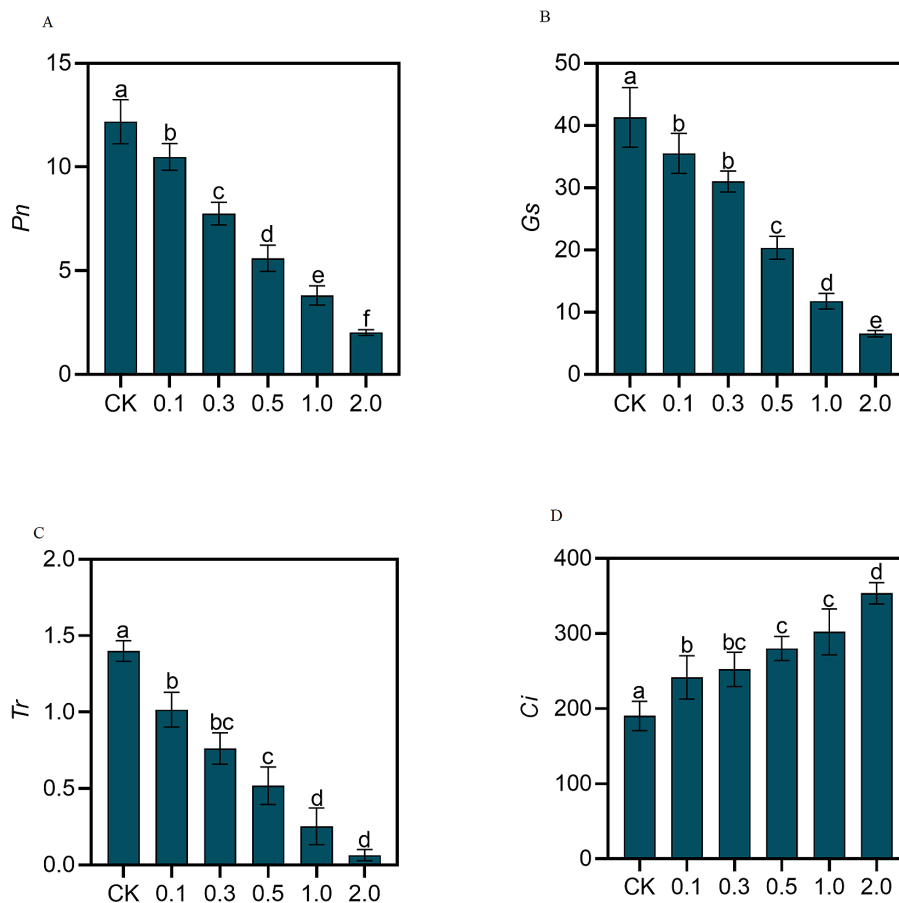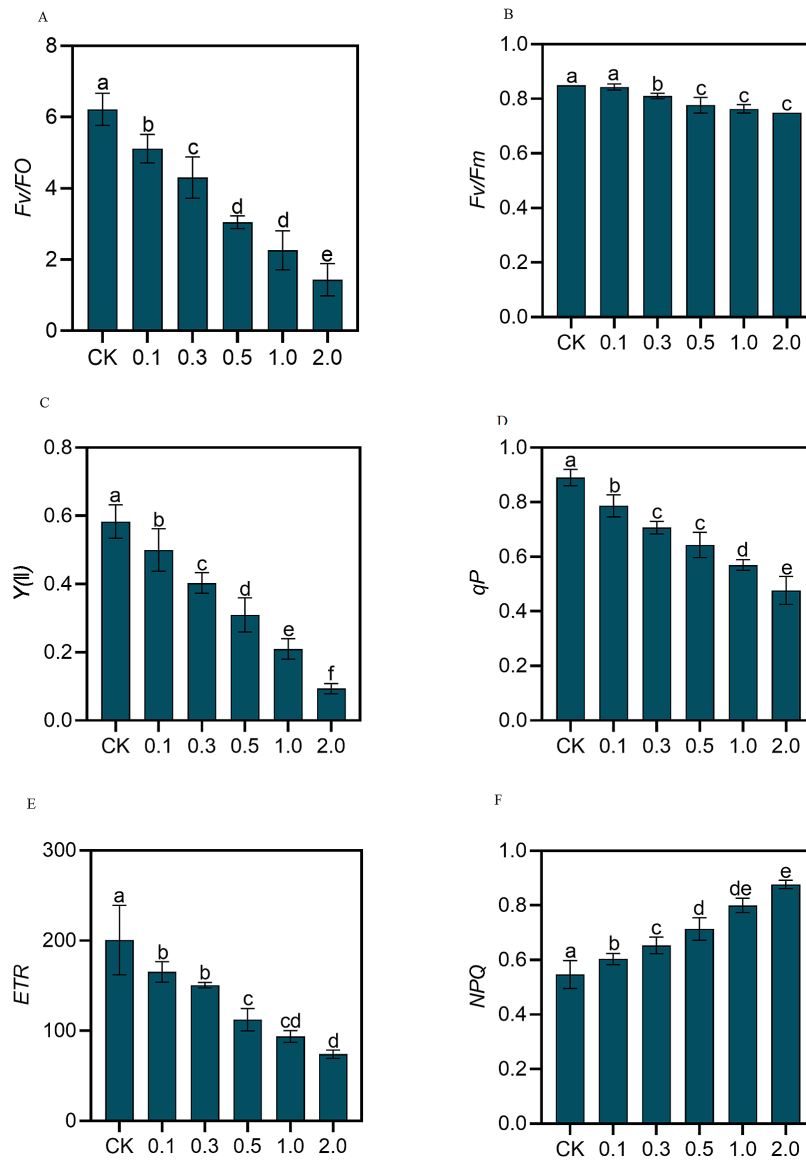
Figure 3. (a) Pn, (b) Gs, (c) Tr, (d) Ci. Duncan was used to analyze the mean values of each parameter, and the significant difference between treatments marked with different lowercase letters was at P < 0.05.

63.1% and 46.4% respectively, while NPQ was notably differed from the control, with an increase of 37.6%. Studies have reported that Fv/Fm is the response of plants to photoinhibition under stress, and serving as the largest indicator of original light energy capturing. Generally, Fv/Fm ratio decreases when plants experience stress (Sobiech et al., 2020), In this experiment, when the residual atrazine amount was not less than 0.1 mg·kg^{-1}, Fv/Fm decreased, this indicates that after lucern received stress from atrazine, the leaves suffered photoinhibition or the PSII complex was damaged, which is consistent with studies of other plants damaged by herbicides (Weber et al., 2017). Fv/Fo is a parameter in the photosynthetic electron transport chain, and its decrease indicates damage to the main part of the PSII reaction center (Borawska-Jarmułowicz et al., 2014).

The decline in Y(II) and ETR showed that the actual photochemical quantum yield and noncyclic photosynthetic electron flow rate of PSII photosynthetic reaction center were significantly reduced (Park et al., 2017), which agreed with previous research findings (Chen et al., 2016). The study demonstrated a significant reduction in qP, indicating a decrease in the proportion of the open part of the PSII reaction center and a decline in photosynthetic efficiency. Electron transfer was impeded under atrazine stress (Zhang et al., 2015). NPQ elevation represents alfalfa's response to atrazine stress, serving to prevent excessive light energy from causing damage to the photosynthetic structures. This aligns with findings from prior studies (Pimentel, 2014; Liu et al., 2015).

The correlation analysis between gas-exchange and chlorophyll fluorescence indicators of lucern reveals significant relationships. NPQ in alfalfa exhibits an extremely significant negative correlation with Pn, Gs, Tr, Fv/Fo, Fv/Fm, qP, Y(II), and ETR (P<0.01), while demonstrating a highly significant positive correlation with Ci (P<0.01) (Table 2). Ci showed a positive correlation with Fv/Fo, Fv/Fm, qP, Y(II), and ETR (P<0.01). SPAD, Pn, and Gs exhibited an extremely negative relation with Ci (P<0.01), and an extremely positive relation with Tr, Fv/Fo, Fv/

Figure 4. Effect of atrazine residue on chlorophyll fluorescence parameters of lucern. (a) Fv/Fo, (b) Fv/ Fm, (c) Y(II), (d) qP, (e) ETR, (f) NPQ. Duncan was used to analyze the mean values of each parameter, and the significant difference between treatments marked with different lowercase letters was at P < 0.05

Table 2. Photosynthetic features and chlorophyll-related indicators of lucern correlation analysis

Variable	SPAD	Pn	Gs	Tr	Ci	Fv/Fo	Fv/Fm	qP	Y(Ⅱ)	ETR	NPQ
SPAD	1.000										
Pn	.997	1.000									
Gs	.992	.989	1.000								
Tr	.989	.992	.980	1.000							
Ci	-.974	-.966	-.966	-.966	1.000						
Fv/Fo	.995	.997	.993	.994	-.976	1.000					
Fv/Fm	.988	.994	.988	.982	-.939	.990	1.000				
qP	.996	.995	.985	.993	-.986	.996	.980	1.000			
Y(Ⅱ)	.998	.994	.991	.982	-.982	.993	0.983	.996	1.000		
ETR	.988	.991	.990	.993	-.975	.998	.984	.992	.986	1.000	
NPQ	-.993	-.983	-.991	-.970	-.979	-.984	-.972	-.986	-.996	-.977	1.000

Fm, qP, Y(II), and ETR (P<0.01). These results indicate that as the damage to the PSII structure and function of lucern leaves intensifies, the efficiency of PSII light energy conversion and photochemical electron transfer activity diminishes. This impact hinders the photosynthesis of lucern, leading to impaired development and a significant reduction in production potential. Therefore, in the current stage of agricultural development, it is imperative to strictly control or replace herbicides. Additionally, reasonable arrangements such as crop rotation and appropriate intervals between crops should be implemented to mitigate the impact on subsequent crops, thereby minimizing ecological environmental pollution.

CONCLUSIONS

Current research indicates that an excessive amount of atrazine residue in the soil hinders the growth of lucern, leading to stunted and yellowed leaves, and a consequent reduction in the SPAD value. Experimental findings further illustrate that an excess of residue influences alterations in the photosynthetic characteristics of lucern, such as fluctuations in Pn, Gs, Tr, Ci, and changes in chlorophyll fluorescence indicators, comprising variations in Fv/Fo, Fv/Fm, qP, Y(II), ETR, and NPQ. These alterations ultimately diminish the production potential of lucern, resulting in phytotoxicity to subsequent crops and environmental pollution. The analysis of relevant results highlights that damage to the photosystem correlates with a reduced growth rate of lucern.

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