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STUDY ON TOXICOLOGICAL EFFECTS OF HERBICIDE ATRAZINE ON ALFALFA SEEDLINGS

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Atrazine is a pre and post seedling herbicide that selects internal absorption conduction. Due to its high efficacy, low dosage, wide herbicide spectrum, and long residual efficacy period, it has been rapidly and widely used and promoted. From a global perspective, atrazine is currently one of the most widely used herbicides. However, due to its extensive use, it has been found to cause pesticide damage in soil and the environment. In order to study the effects of different concentrations of atrazine on the growth and physiological effects of alfalfa seedlings, six different concentrations of atrazine (0, 0.1, 0.2, 0.4, 0.8, 1.6 mg/L) were used to treat alfalfa seedlings using the pre seedling soil treatment method after indoor sowing. The growth and chlorophyll, malondialdehyde (MDA) content of alfalfa seedlings were measured. Firstly, determine the effect of atrazine on the growth of alfalfa. As the concentration of atrazine treatment increased, compared with the variant without treatment, the length of the aboveground and underground parts showed a significant inhibitory effect. Under the 1.6 mg/L treatment, the length of the aboveground part decreased by 26.3%, while the length of the underground part decreased by 39.5%. At the same time, it was found that the dry weight of alfalfa also showed a significant decreasing trend. At the maximum treatment concentration, the dry weight of the aboveground and underground parts decreased by 20.0% and 14.3%, respectively, The residue of atrazine in soil affects the growth and development of plants. The main mechanism of action of atrazine is to inhibit photosynthesis and hinder carbohydrate and photochemical synthesis reactions, preventing normal plant growth. The experiment found that as the concentration increased, the chlorophyll content in alfalfa leaves gradually decreased. Compared with the variant without treatment the chlorophyll content decreased by 48.0%. At a concentration of 1.6 mg/L, the chlorophyll content decreased by 20.9% compared to alfalfa seedlings without treatment. At the same time, the TBARS determination results showed that the content of MDA was the highest at a concentration of 0.8 mg/L, which was 2.50 times higher than on the control. However, 1.6 mg/L is the lowest, only 4.32 times that of with the variant without treatment. Atrazine causes membrane lipid peroxidation in alfalfa seedlings, leading to increased lipid permeability and toxicity at high concentrations. Therefore, attention should be paid to controlling the concentration of atrazine in production, and the dosage should not be arbitrarily increased to avoid causing drug harm.

***Key words**: atrazine, alfalfa seedling, growth, toxicity.*

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Introduction. Atrazine is a triazine herbicide widely used worldwide, which can control annual *Gramineae* weeds and broad-leaved weeds, and also has a certain inhibitory effect on some perennial weeds (Iriel et al., 2014; Kumar et al., 2016; Zhao et al., 2017). It is the second herbicide in the world, with an annual consumption of about 70 000–90 000 tons (Chevrier et al., 2011; Sass & Colangelo, 2006; Kumar et al., 2013; Cheng et al., 2016). It was first introduced in the 20th century and is usually used alone or in combination with other herbicides for agricultural applications. Because of its low cost and practical and effective nature, it is widely used in agricultural practices (Correa et al., 2007; Kadian et al., 2008; Lewis et al., 2009).

The main mechanism of atrazine is that it blocks the carbohydrate and photochemical synthesis reaction by inhibiting photosynthesis, and changes the growth, enzyme process and photosynthesis of plants (Hess, 2000; Qian et al., 2014; Karlsson et al., 2008). However, a large amount of atrazine is applied during planting, resulting in its residues in soil, groundwater and crops (Arora et al., 2008; Sui & Yang, 2013). According to the latest reports the concentration of atrazine detected in agricultural areas is 42 μg/L, the detection concentration in the river basin is 102 μg/L (Kolpin et al., 1998; Powell et al., 2011, Xing et al., 2015), so it becomes a toxic pollutant in the environment (Sullivan et al., 2009; Jablonowski et al., 2011). The widespread atrazine pollution and its toxicity have received widespread attention, especially for researchers who study its ecotoxicology and seek to pollute soil and plant or crop biodegradation by organic chemicals (Gerhardt et al., 2009; Zhang et al., 2016).

Alfalfa is one of the most important legume crops in the world. Due to its high nutritional quality and strong adaptability to the planting area, it has great biological value (Li et al., 2011; Yang et al., 2022). In addition, its root system has the ability of nitrogen fixation, which can increase the content of soil organic matter and improve soil physical and chemical properties (Salas et al., 2017). The widespread use of atrazine in soil and the residue of atrazine are likely to affect the growth of alfalfa. It is reported that one year after the application of atrazine in the corn field, it may still have an impact on the growing atrazine sensitive crops (Zhang et al., 2012; Zhang et al., 2014; Besplug et al., 2004), thus further affecting the quality of dairy products and livestock products. Therefore, it is of great significance to study the toxicological effects of atrazine in alfalfa for the development of environment and agriculture.

Therefore, the purpose of this study was to examine the effect of different concentrations of atrazine on biomass, chlorophyll and malondialdehyde content in alfalfa.

Materials and methods. *Atrazine treatment and plant treatments.* Atrazine was purchased from Syngenta, Henan Province, China, with purity of 99.47%. Alfalfa seeds were provided by the Institute of Plant Protection, Henan Academy of Agricultural Sciences, China. The test soil was collected from the test site of Henan Academy of Agricultural Sciences. The evenly seeded alfalfa is sterilized with distilled water. The germination and planting methods refer to Zhang (Zhang et al., 2014), and then the seedlings with consistent growth were transferred to dark plastic cups, where 10 seedlings were planted in each plastic cup. The concentration of atrazine treatment was 0, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/L, respectively. The seedlings were cultured in a growth box under the following conditions: light intensity – 200 umol (m^2 s), illumination time – 14h/10h (day/night), and cultivation temperature – 25^0C/20^0C. During the experiment, each treatment was repeated 3 times, and cultured for 10 days (biomass measurement) and 6 days (chlorophyll and MDA).

Determination of biomass and physiological indexes of alfalfa seedlings. The alfalfa was washed and dried, and the biomass and physiological indexes of plants were measured. Among them, the content of chlorophyll was determined by acetone method, referring to the methods of Yin et al. (2008) and Zhang et al. (2017), the content of malondialdehyde – by the content of thionenenebb barbituric acid reactants (TBARS), referring to the method of Zhang et al. (2021).

Data analysis. The test data is represented by mean ± standard deviation. The measured data were analyzed by DPS software for single-factor significance, and Duncan, s new complex range method was used to compare the indicators of significant difference. The histogram was drawn by Graphpad software (Version 6.01, IBM) (Zhang et al., 2017).

Results. *3.1. Effect of atrazine on alfalfa biomass.* In order to study the toxicological effect of atrazine on alfalfa, firstly, the effect of atrazine on alfalfa biomass was measured. Alfalfa growth was strongly inhibited after atrazine treatment for six days compared to the control (Fig. 1). With the increase of atrazine treatment concentration, the elongation and the biomass of the shoot and the root gradually decreased. When treated with 0.10 mg/L atrazine, the elongation of the shoot and the root were 26.3% and 40.0% of the variant without treatment, respectively (Fig. 1). Compared with elongation, atrazine has a more severe effect on alfalfa biomass. At the same treatment concentration, shoot and root biomass was 20.0% of the control variant. Under the treatment of 0, 0.1, 0.2, 0.4, 0.8, 1.6 mg/L atrazine series concentrations, significant changes can be seen after the growth of alfalfa for 6 days. Length of the shoot and the root decreased significantly compared with the variant without treatment. Under the treatment of 1.6 mg/kg concentration, the length of the shoot decreased by 26.3%, while the length of the shoot decreased by 39.5% (Fig. 1).

Atrazine had a more serious effect on alfalfa biomass compared with elongation. Under the same concentration treatment, the trend of shoot and root length is similar, and plant tissue biomass also showed a significant decreasing trend. At the maximum treatment concentration, the biomass of the shoot and root decreased by 20.0% and 14.3% respectively (Fig. 2).

3.2. Effect of atrazine on chlorophyll content of alfalfa. In order to detect the physiological response of alfalfa seedlings to atrazine, we measured the chlorophyll content in the leaves of alfalfa seedlings. As can be seen from Fig. 3, the chlorophyll content gradually decreased with increasing concentration of atrazine treatment. At the treatment concentration of 1.6 mg/l, the chlorophyll content decreased by 20.9% compared to alfalfa seedlings without atrazine.

3.3. Effect of atrazine on the content of MDA in alfalfa. The content of MDA is the most commonly used indicator to indicate the degree of membrane lipid peroxidation of plants under external stress. It is reported that under the treatment of pesticides, plants are induced to produce reactive oxygen species, which causes membrane lipid peroxidation of cells and increases the content of MDA in plant tissues. As shown in Fig. 1, the content of MDA in the aerial part and root of alfalfa increased after treatment with atrazine at different concentrations for 6 days. When the treatment concentration was 0.8 mg/L, the MDA content in the aboveground part and root were reaching the maximum value, which were 9.06 and 5.96 nmol/(gFW), respectively, 2.50 and 4.32 times higher than control. When the concentration of atrazine was 1.6 mg/L, the content of MDA in alfalfa tissue decreased significantly compared with 0.8 mg/L. This may be due to the fact that excessive atrazine disrupts the production and clearance mechanism of intracellular reactive oxygen species, and causes serious oxidation of plant membrane lipids, resulting in a decline in MDA content.

As a herbicide, atrazine is widely used in various plants. In the process of growth and development, atrazine stress will show different degrees of growth retardation, and also inhibit photosynthesis, which can be reflected by relevant physiological and biochemical indicators (Wan et al., 2006). Under stress, receptor proteins in plants transmit stress signals and produce a large number of reactive oxygen species. When the induced reactive oxygen species are excessively accumulated in cells or the antioxidant system in cells is defective, it will stimulate the production of free radical chain reaction and cause membrane lipid

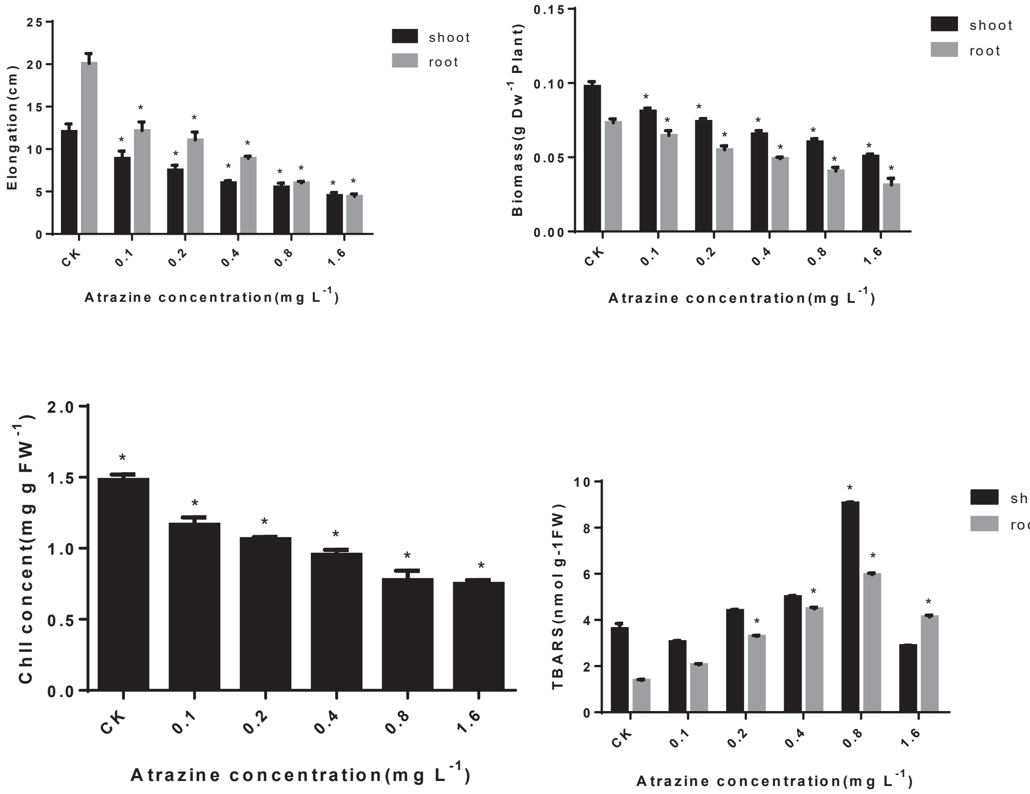

Fig. 1. The effect of atrazine on various indicators: on the elongation (first row, left), on the biomass of the shoot and the roots (first row, right), on chlorophyll content (second row, left), on MDA content (second row right). Values are the means ± SD (n = 3). Asterisks indicate significant differences between the treatments and the control (P < 0.05)

peroxidation, resulting in toxic effects in plants. For example, plant biomass, chlorophyll content, and membrane lipid peroxidation decrease (Niansheng et al., 2006).

A small amount of atrazine can inhibit its growth and chlorophyll content at a concentration of 1 μg/kg, a slight photosynthetic attenuation can be observed in *Cylotella menechiniana* within 5 minutes (Millie and Hersh, 1987). *Scenedesmus quadricaudata* and *Microcystis aeruginosa* are not sensitive to atrazine, and atrazine has a significant inhibitory effect on the growth of the two algae, and low concentration of atrazine can also inhibit their growth (Xu et al., 2008). Xia et al. (2020) reported that with the increase of atrazine concentration, atrazine treated gladiolus showed symptoms of low growth and yellow leaves, and chlorophyll content showed a downward trend, which is consistent with the results of this experiment. Liu et al. (2017) found that under the action of atrazine, the length, weight and quantity of main root and lateral root of soybean seedlings decreased to varying degrees, indicating that atrazine has a certain toxic effect on soybean, which is consistent with the results of the toxicological effect of atrazine on plants in this experiment.

With the increase of concentration, the toxic effect of atrazine on aquatic vascular plants increased correspondingly. The experiment showed that the concentration of bitter grass is 100 μg/L after exposure to atrazine for six weeks, 29% of growth was inhibited, at 320 μg/L. Under the stress of g/L atrazine, 36% of the growth was inhibited, after 5 weeks of exposure, if the concentration is 3.2 μg/L, 1% growth was inhibited, and the concentration is 100 μg/L, 36% of the growth was inhibited (Mukherjee, 2019). *Lysimachia* has a high tolerance to atrazine at low concentration, but it were obviously damaged by atrazine under the treatment of 16 mg/L (Wang et al., 2011). At the same time, it was also found that under atrazine stress, the fresh weight and chlorophyll content of shallot decreased to varying degrees, and the root activity and activity decreased (Wang et al., 2011). Under the treatment of atrazine at medium and high concentrations, the biomass and a series of physiological and biochemical indicators of *Phyllostachys chinensis* were also greatly affected (Zhang et al., 2021). The residual amount of atrazine in farmland will be toxic, and will also be toxic to subsequent crops such as rice, wheat, soybean, and sugar beet. Among them, atrazine is more toxic to rice (Wang Yingzi et al., 2002). Previous studies on the physiological indicators of alfalfa mycorrhizal symbiosis under atrazine stress have found the accumulation and growth indicators of alfalfa at different time periods at the same concentration gradually decrease, and the chlorophyll content gradually decreases (Zhang et al., 2016), which is consistent with the results of this experiment.

Atrazine is an inhibitor of photosynthesis. Large amounts of atrazine can remain in the soil, affecting chlorophyll content and leading to the production of a large amount of reactive oxygen species. It is an important intermediate that damages cell structure lipids, membranes, proteins, and nucleic acids, and leads to membrane lipid peroxidation. Membrane lipid peroxidation is an important cause of plant oxidative damage, increasing the permeability of plant membrane lipids and damaging membrane structure and function, ultimately, it causes damage to plants and is extremely harmful to plant cells (Fan et al., 2018). Therefore, rational medication should be used in production, and the dosage should not be arbitrarily increased to avoid drug harm. We can vigorously cultivate herbicide resistant varieties or mix them with other safety agents and additives to reduce the harm of atrazine to subsequent crops.

Conclusions. In this experiment, the toxicological effects of atrazine at different concentrations on alfalfa seedlings were established. The toxic effects of atrazine at different concentrations on the growth of alfalfa seedlings were analyzed by measuring different indicators of alfalfa seedling biomass, chlorophyll content and MDA content. The results showed that under the treatment of 0.1–1.6 mg/L atrazine concentration, the growth of alfalfa seedlings was significantly inhibited, including decrease in shoot and root elongation and biomass. Atrazine had a more serious impact on the biomass of alfalfa. Under the same treatment concentration, the biomass of shoot and root was 28.0% and 31.1% of that of the control, respectively. Significant changes can be observed after 6 days of alfalfa growth. Among them, compared to variant without treatment, the length of the shoot and the root significantly decreases. Under the concentration of 1.6 mg/L, the length of the shoot decreased by 26.3%, while the length of the root decreased by 39.5%. At the same time, the chlorophyll content gradually decreased, and at a concentration of 1.6 mg/L, compared with alfalfa seedlings without atrazine application, the chlorophyll content decreased by 20.9%. MDA content is the most commonly used indicator of membrane lipid peroxidation in plants under external stress. After 6 days of treatment with different concentrations of atrazine, the MDA content in the shoot and roots of alfalfa increased. When the treatment concentration is 0.8 mg/L, the MDA content in the shoot and roots reaches its maximum value.

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Дослідження токсикологічного впливу гербіциду атразину на проростки люцерни

Атразин – один із найпоширеніших гербіцидів для боротьби з бур'янистою рослинністю. Він має до- і післясходову дію та впливає на внутрішню абсорбційну провідність. Завдяки високій ефективності, низькій дозі, широкому спектру дії гербіциду та довгому залишковому періоду ефективності він швидко та широко використовується та пропагується. Через його широке використання він став серйозним забруднювачем ґрунту та води. Щоб оцінити вплив атразину на бобові, у цьому експерименті виміряли різні показники біомаси, вмісту хлорофілу та вмісту малонового діальдегіду (МДА) для аналізу токсичної дії атразину в різних концентраціях на ріст проростків люцерни. У дослідженні вивчили атразин виробництва Syngenta. Рослини проростили за наступних умов: інтенсивність світла 200, час освітлення – 14/10 годин (день/ніч), температура для вирощування 20–25°С. Повторення трьохкратне. Вивчили наступні показники концентрації атразину: 0, 0,1, 0,2, 0,4, 0,8 та 1,6 мг/л. Результати показали, що порівняно з варіантом без обробки визначений індекс токсичності біомаси та вміст хлорофілу були в основному однаковими, що показало очевидний інгібуючий вплив атразину на ріст проростків люцерни зі збільшенням концентрації застосування. При обробці 1,6 мг/л довжина надземної частини зменшилася на 26,3%, довжина підземної – на 39,5%, вміст хлорофілу – на 48,0%. Суха маса рослинної тканини також продемонструвала значну тенденцію до зменшення. За максимальної концентрації обробки суха маса надземної та підземної частин зменшилась, відповідно, на 20,0% та 14,3%. Було відмічено поступове зменшення вмісту хлорофілу. За концентрації 1,6 мг/л цей показник в проростках люцерни знизився на 20,9% порівняно з варіантом без атразину. Результати вимірювання МДА показали, що його вміст був найвищим при концентрації 0,8 мг/л, що в 2,5 рази перевищило варіант без обробки. За концентрації 1,6 мг/л було виявлено найменшу кількість діальдегіду, що у 4,32 перевищило показник у варіанті без обробки. Атразин викликав мембранне перекисне окислення ліпідів у проростках люцерни, що призвело до підвищення ліпідної проникності та токсичності у високих концентраціях.

Ключові слова: *атразин, проростки люцерни, ріст, токсичність.*