Potential Allelopathic Interference of *Abutilon theophrasti* Medik. Powder/Extract on Seed Germination, Seedling Growth and Root System Activity of Maize, Wheat and Soybean

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Abstract: The velvetleaf (*Abutilon theophrasti* Medik.) is a strong and competitive weed in fields that inhibits the growth of crops. Reports have suggested that allelopathy is one of the reasons for this inhibition; however, the mechanism of this allelopathy remains unclear. In this study, velvetleaf powder/extracts were shown to inhibit seed germination, growth and yield in maize, wheat and soybean through petri dish, pot and field control experiments. We observed a concentration-dependent inhibition of the seed germination rate for all three crops. The root tip structure changed significantly and the embryo even died when irrigated with a high concentration of the extract (10 mg·mL⁻¹). After adding velvetleaf powder, the malondialdehyde (MDA) content in crop seedlings was dose-dependent, and the superoxide dismutase (SOD) activity of maize, wheat and soybean showed the maximum values under treatment with 1.25, 5 and 5 mg·cm⁻³, respectively. The activity of peroxidase (POD) showed the highest value under the 5 mg·cm⁻³ treatment in maize and wheat seedlings and under 10 mg·cm⁻³ treatment in soybean seedlings. However, sugar, protein content and root activity in all three crops was the lowest under the 10 mg·cm⁻³ treatment. Therefore, velvetleaf may decrease the productivity of three crops by changing the antioxidant enzyme activities, root system activities and root tip structures.

Keywords: velvetleaf; crops; allelopathy; antioxidant enzymes; root system activity

1. Introduction

Velvetleaf (*Abutilon theophrasti* Medik.) is an annual subshrub herb belonging to the *Abutilon* genus in the mallow family. It is distributed throughout Asia, Europe and North America. In China, except for the Qinghai Tibetan Plateau, velvetleaf is produced in all regions as it has a wide distribution in northeast China [1]. Velvetleaf is recognized as a strong and competitive main weed in fields that inhibits the yield and biomass of crops such as maize (*Zea mays* L.) [2], soybean (*Glycine max* L.) [3], wheat (*Triticum aestivum* L.) [4], cotton (*Gossypium hirsutum* L.) [5] and sorghum (*Sorghum bicolor* (L.) Moench) [6] etc. It is important to clarify the interference mechanism of velvetleaf in crops.

Research has shown that weeds compete with crops for resources such as light, nutrition, space and water [7], and are considered as field pests that inhibit crop growth
and reduce the productive potential of these crops. Their existence in farmland reduces
the quantity and quality of agricultural products and causes huge economic losses to
farmers [8]. Many weed species interfere with crop growth through allelopathy [9]. Plant
allelopathic mechanisms mainly include the effect on the permeability of organelles [10],
the root tip structure [11], enzyme function and activity [12], protein synthesis and gene ex-
pression [13], etc. Wheat can detect chemical signals from the root exudates of weeds such
as velvetleaf and then increase allelopathy in response. The allelopathic influence of wheat
on weeds depends not only on the specific weeds but also on the root interactions [14].
Elmore [15] found that aqueous extracts of velvetleaf had an inhibitory effect on the growth
of turnip (Brassica rapa L.). Some researchers have reported that velvetleaf can impose
its allelopathy on cress (Lepidium sativum L.), and the intensity of this allelopathy was
significantly affected by drought stress and temperature [16]. A study by Huo [17] showed
that an aqueous extract of velvetleaf inhibited the germination and growth of Lactuca
sativa L., Portulaca oleracea L., and Amaranthus retroflexus L. There are fewer reports on its
allelopathic effects on maize, wheat and soybean. Craig et al. [18] studied the allelopathic
effect of velvetleaf leaves on soybean and found that several known allelopathic substances
in velvetleaf leaves inhibit photosynthesis and may play a role by interfering with the
relationship between plants and water. Konstantinovic et al. [19] showed that extracts of
Xanthium strumarium Patrin ex Widder and Abutilon theophrasti Med. derived with different
leaching agents and at different concentrations had different inhibition effects on soybean
and maize. However, the effects on the antioxidant activity, lipid peroxidation levels, the
root system activity and the root tip structure of crops have not been reported in detail.

In the antioxidant protection system, various antioxidant enzymes help to clear su-
peroxide dismutase (ROS) in the body in different ways and play different roles [20].
Peroxidase (POD) is widely present in plants and animals, microorganisms and cultured
cells as a kind of oxidoreductase. It can catalyze H2O2 oxidation of phenol and amine
compounds with the elimination of hydrogen peroxide, phenol and amine toxicity in a dual
role. In this study, POD enzyme activity was determined by the guaiacol method according
to Fatemi [21]. As the first line of defense of the biological antioxidant system, superoxide
dismutase (SOD) is the most important member of antioxidant enzyme system and is also
the main generating enzyme of H2O2. Szőke’s method [22] was used to determine SOD
activity by the nitro blue tetrazole reduction method to reflect the antioxidant activity
of the body. Phospholipids in biofilm lipids are rich in polyunsaturated fatty acids. In
the presence of O2, they are easily attacked by free radicals and their active derivatives
produce lipid peroxides, which are gradually decomposed into a series of complex
compounds. Many aldehydes, such as malondialdehyde (MDA), are produced by the β
break of lipoxy groups [23]. In this study, the content of MDA in biological samples was
determined by barbituric acid staining with Duarte’s method [24] to reflect the level of lipid
peroxidation in crops.

The accumulation of sugar in plants is related to the osmotic pressure of cells, and
soluble protein content is an important indicator used to understand the total metabolism
of plants [25]. Therefore, Coomassie bright blue G-250 staining and the anthrone colorimetric
method were used to determine the content of sugar and protein, respectively, as these are
important physiological and biochemical indicators of plants, according to Chen [26] and
Wu’s [27] methods.

Root growth and activity directly affect the nutrient status, yield and biomass lev-
els of aboveground parts [28]. The reduction method of triphenyltetrazole ammonium
chloride (TTC) was used to detect seedling root activity, which could better charac-
terize root absorption activity under different treatments [29]. Positive fluorescence
microscopy [30] was used to observe the root tip structure and reflect the growth status
and physiological characteristics of crops. Thus, the mechanisms of these aspects still
need to be studied further.

Maize, soybean and wheat are important crops in dry farmland. Velvetleaf is one
of the main weeds in the field, so it is necessary to study the inhibition mechanism of
velvetleaf on these three crops in order to manage and control velvetleaf in farmland. This study was conducted to explore the allelopathy mechanism of velvetleaf powder or an aqueous extract on maize, soybean and wheat by investigating the effects on the growth and yield, antioxidant enzyme activity, lipid peroxidation levels, root system activity, and root tip structure of these three crops.

2. Materials and Methods

2.1. Materials and Chemicals

The velvetleaf plants were harvested in September 2019 in Kangrong Town, Lanxi County, Suihua City, Heilongjiang Province. The leaves were cleaned and air-dried for two weeks, then ground into a fine powder and stored in a refrigerator at 4°C for further study. The maize, wheat and soybean varieties were “Liaodan 120”, “Kenong 28” and “Longfu 20”, respectively, and were purchased from the seed market of Heilongjiang Province (Harbin, Heilongjiang Province, China).

All chemicals were analytical grade, and all were supplied by BioBest Company (Chengdu, Sichuan Province, China). Distilled water was prepared with multifunctional ultra-pure water equipment (Unique LC R20, Xiamen Ruisijie Water Purification Technology Co., Ltd., Xiamen, Fujian Province, China).

The soil was loam with a pH of 6.86, the organic matter content was 34.6 g·kg⁻¹, total N was 1.83 g·kg⁻¹, alkali-dissolvable N was 163.2 mg·kg⁻¹, total P was 0.57 g·kg⁻¹, available P was 28.6 mg·kg⁻¹, total K was 21.3 g·kg⁻¹ and available K was 183.6 mg·kg⁻¹.

2.2. Preparation of the Velvetleaf Water Extract

Velvetleaf powder (100 g) was taken and soaked in 1 L of distilled water at 25°C for 30 min. During soaking, an ultrasonic cleaning machine (KQ-250DB, Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, China) was used to assist extraction. The ultrasonic frequency was set at 35 kHz, the power was set to 500 W and the temperature was set to 25°C. The crude extracts were centrifuged (LXJ-IIB Shanghai Anting Scientific Instrument Factory, Shanghai, China) at 4000 rpm, then the velvetleaf water extract with a concentration of 10 mg·mL⁻¹ was extracted from the supernatant and diluted with distilled water to obtain 5 mg·mL⁻¹, 2.5 mg·mL⁻¹ and 1.25 mg·mL⁻¹ water extracts, which were stored in a refrigerator at 4°C. The velvetleaf extracts were used to analyze the inhibition of the germination rate and the root tip structure of soybean, wheat and maize in a petri dish experiment.

2.3. Test Method

Petri dish and pot cultivation methods combined with field experiments were adopted. The petri dish experiment was conducted at the Key Laboratory of Forest Plant Ecology of the Ministry of Education. The pot experiment site was located in the greenhouse (45°43′8″ N, 126°38′3″ E) of the Key Laboratory of Forest Plant Ecology of the Ministry of Education, Northeast Forestry University. The field experiment was conducted in Kangrong Town (46°09′57″ N, 126°09′6″ E), Lanxi County, Heilongjiang Province from the beginning of April to the end of September 2020.

2.3.1. Petri Dish Experiment

All seeds were surface-sterilized with 0.5% potassium permanganate for 5 min before washing in distilled water. The seeds were evenly placed in petri dishes (d = 90 mm) lined with two layers of Whatman No.1 filter paper with 10 seeds per dish; 10 mL of the velvetleaf water extract at different concentrations was added, with distilled water as the control (CK). Each treatment was repeated six times. The concentration of velvetleaf extract was set to 1.25 mg·mL⁻¹, 2.5 mg·mL⁻¹, 5 mg·mL⁻¹ and 10 mg·mL⁻¹. The petri dishes were placed in an environmental chamber at temperatures of 25°C/15°C (day/night) and 60% relative humidity. Germinated seedlings (radicle protruded by ≥2 mm) were counted at 12 h intervals, until cumulative germination levelled off (seven, seven and five days for maize, wheat and soybean, respectively). The growth status of the three crops
treated with different concentrations of velvetleaf extract was observed. On the eighth
day of germination, the seedlings were removed from the petri dish to preserve the roots’
integrity, and the radicle length of the three crops was measured with a vernier caliper,
for which five seeds were randomly selected from each petri dish for measurement, and
the average value was calculated. After the measurement, 2 mm of the distal taproot was
removed with disinfected scissors for subsequent analysis of the apical microstructure.
The inhibition rate of velvetleaf extract on the seeds was calculated using the equation
given below [31]:

\[ Y = \left[ (C - T) / C \right] \times 100 \]

where Y is the inhibition rate (%), C is the germination rate of the control (%) and T is the
ermination rate of the treatment (%).

2.3.2. Pot Experiment

Potted soil was collected from the ploughed layer (0–20 cm) in the experimental field
of Kangrong Town, Lanxi County. The mixture of 1460 g screened soil (≤ 2 mm) and
velvetleaf powder was placed in a plastic pot (d = 20 cm). Four treatments (1.25 mg·cm\(^{-3}\),
2.5 mg·cm\(^{-3}\), 5 mg·cm\(^{-3}\) and 10 mg·cm\(^{-3}\) of velvetleaf powder) were established in the
pots, and the soil without the velvetleaf powder was used as the control. Maize seeds
were sown in the pots on 20 April 2020, with two seeds in one hole and three holes in
total, for which the planting depth was 2–3 cm. These were later thinned to three maize
seedlings at the same growth stage in each pot. Wheat seeds were evenly sown into
the pot on 5 April 2020, with three seeds in one hole and a total of 10 holes, at a sowing
depth of 3–4 cm. Ten wheat seedlings at the same growth stage were kept in each pot.
Soybean seeds were planted in the soil on 20 April 2020, with three holes in each pot and
two seeds in each hole, which were covered with soil 2 cm thick, followed by thinning
to three soybean seedlings at the same growth stage in each pot. In the greenhouse, the
temperature was 20–25 °C during the day and 18–20 °C at night. The relative humidity
was 30–80% with natural illumination. During crop cultivation, according to the principle
of heat preservation, the greenhouse was properly ventilated to prevent high temperatures,
humidity and diseases. All treatments were repeated three times. After 20 days of growth,
the seedlings of the three crops were dug up and washed, and then the aboveground and
underground biomass; the activity levels of antioxidant enzymes (POD, SOD); the contents
of MDA, sugar and protein; and root activity were analysed.

2.3.3. Field Experiment

Kangrong Town, Lanxi County, has a cold temperate semihumid monsoon climate
with four distinct seasons, as well as the rainy and hot seasons. The average monthly
temperature and precipitation were 12.4–23.2 °C and 98 mm in the whole growth stage
of the three crops. The main grain crops were maize, wheat and soybean. The test area is
flat, and no crop production had been carried out in the previous three years. Each plot
was managed by the same techniques, and pesticides were not used for pest control. To
ensure that the maize, wheat and soybean thrived, the soil was kept moist throughout the
growing season. In addition, weeding machines were used to strictly control weeds and
reduce interspecific competition.

The field experiment consisted of five treatments, in which 0 kg·m\(^{-2}\), 0.25 kg·m\(^{-2}\),
0.5 kg·m\(^{-2}\), 1 kg·m\(^{-2}\) and 2 kg·m\(^{-2}\) of velvetleaf powder, respectively, was uniformly
scattered across the surface of the soil. A random block design was used in the field, with
three replicates, and the plot area was 27 m\(^2\) (6 m × 4.5 m). The plant spacing of maize,
wheat and soybean was 35 cm, 15 cm and 10 cm, and the row spacing was 55 cm, 40 cm
and 20 cm, respectively.

The biomass (dry weight per plant) of maize, wheat and soybean in the field was
measured throughout the whole growing period of the three crops (see Table 1 for de-
tails). During the sampling process, representative healthy plants with similar growth and
complete leaves under different treatments were selected from each plot and dug up with
the roots. Ten plants were collected from each plot, and this was repeated three times, for a total of 30 plants. After drying at 105 °C for 60 min and at 85 °C until a constant weight, the average dry weight per plant (g plant\(^{-1}\) DW) of each crop was measured by weighing. After the crops were mature, the seeds were dried and hung in an air-drying shed until the moisture content was 14% and weighed to calculate the average yield per plant (g plant\(^{-1}\) DW) of the three crops.

Table 1. Field sampling periods for maize, wheat and soybean.

| Crops | Sampling Stage (Date) |
|-------|-----------------------|
| Maize | Emergence stage (May 5) |
|       | Jointing stage (June 2) |
|       | Tasseling stage (July 5) |
|       | Silking stage (August 15) |
|       | Maturity stage (September 10) |
| Wheat | Seedling stage (April 20) |
|       | Jointing stage (May 20) |
|       | Heading stage (June 15) |
|       | Filling stage (July 25) |
|       | Maturity stage (August 10) |
| Soybean | Germination stage (May 5) |
|        | Branching stage (June 2) |
|        | Flowering stage (July 5) |
|        | Bulking stage (August 15) |
|        | Maturity stage (September 10) |

2.4. Bioassay

2.4.1. Lipid Peroxidation Assays in Pots

The concentration of malondialdehyde (MDA) was measured by enzyme-linked immunosorbent assay (ELISA) methods using ELISA kits according to instructions of the manufacturers. MDA content was used as an index of lipid peroxidation according to the barbital acid coloring method [20]. Seedling samples of 500 mg were extracted using 2 mL of 1% TCA and centrifuged at 6000 rpm for 10 min, then 1 mL of the supernatant was added to a test tube, 2 mL each of 10% TCA and 0.3% thiobarbituric acid (TBA) was added, and the mixture was heated at 100 °C for 10 min and cooled with water. The absorbance was read by ELISA at 532 nm and 600 nm.

2.4.2. Antioxidant Enzyme Analysis of POD and SOD in Pots

POD and SOD were used as indicators of the seedlings’ antioxidant defense. One unit (U) of POD and SOD activity was expressed as U·g\(^{-1}\)·min\(^{-1}\)·FW\(^{-1}\) and U·g\(^{-1}\)·FW\(^{-1}\). The guaiacol method was applied to analyze the POD enzyme activity [17]. Here, 300 mg of the seedlings was homogenized with 1% TCA and centrifuged at 6000 rpm for 10 min, then 100 µL of the supernatant and 3 mL hydrogen peroxide (containing 0.038% guaiacol)
were placed into a quartz cuvette and immediately transferred to a UV spectrophotometer (UV-2600, Shimadzu Instruments Co., Ltd. Suzhou, China). The absorbance was read every 30 s at 470 nm. SOD activity was measured by the reduction in nitro-blue tetrazolium (NBT) [18]. Here, 300 mg of the seedlings was homogenized with a phosphate buffer (PBS) with a pH of 7.8 and centrifuged at 6000 rpm for 10 min, then 200 µL of the supernatant was transferred into a tube, 3.7 mL NBT was added, and the mixture was centrifuged at 10,000 × g for 20 min. The absorbance was recorded by ELISA at 560 nm.

2.4.3. Protein and Sugar Assays in Pots

Protein contents were determined using Coomassie brilliant blue G-250 staining [23]. Here, 200 mg of the seedlings was homogenized with 10 mL PBS and centrifuged at 6000 rpm for 10 min, then 100 µL of the supernatant was transferred to a tube and added into 100 µL distilled water and 5 mL Coomassie brilliant blue for 2 min to read the absorbance at 595 nm [32]. The content of sugar was analyzed with the anthrone colorimetric method [22]. Here, 0.5 g of the seedlings were homogenized with 10 mL distilled water and centrifuged at 6000 rpm for 10 min, then 100 µL of the supernatant was transferred into a tube and added into 100 µL distilled water and 5 mL anthrone. The mixture was heated at 100 °C for 10 min and cooled with water. The absorbance was read by ELISA at 620 nm.

2.4.4. Root System Activities in Pots

The method of reduced TTC (triphenyltetrazolium chloride) [29] was used to characterize the root activity of crops by calculating the reduction intensity of tetrazolium per unit of root fresh weight. Root tips (500 mg) were cut into small pieces of 1 cm and placed into a tube, and 10 mL TTC and 10 mL PBS were added. The mixture was reacted for 1 h at 37 °C, then 2 mL sulfuric acid was added to stop the reaction. The treated root tips were homogenized with 10 mL ethyl acetate and centrifuged at 6000 rpm for 10 min. The absorbance at 485 nm was read in 2 mL of the supernatant [33]. The root activity of soybean, wheat and maize was calculated according to the following formula:

\[ A = \frac{D}{(1000 \times W \times h)} \]

where \( A \) is the reduction intensity of tetrazole (mg·(g·h)^{-1}), \( D \) is the reduction amount of tetrazole (µg), \( W \) is the root weight (g) and \( h \) is the reaction time (h).

2.4.5. Root Tip Structure Assays in Petri Dishes

To analyze the structural properties, root tips were dyed with 1% sarranine and sliced with a blade and observed by positive fluorescence microscopy (Leica Microsystems GmbH, Wetzlar, Germany). The structure of the root tip was enlarged 100 times for maize and soybean, and 200 times for wheat, and a 50 mg root slice was taken from the 2 mm root tip to analyze the root tip structure [26].

2.5. Statistical Analysis

Statistical analyses were performed with Excel 2013 (Microsoft Corporation, Redmond, WA, USA) and SPSS v.17 statistical software (IBM Crop., Armonk, NY, USA), and the data were imported into Origin (OriginPro8.0, Originlab Corporation, Northampton, MA, USA) to draw the graphics. The data are expressed as the means and standard deviations of three biological replications. Means among different treatments were compared by analysis of variance using the least significant difference (LSD) test at \( p < 0.05 \).

3. Results
3.1. Effect of Velvetleaf Extract on the Germination of Three Crops in Petri Dishes

In the petri dish experiment, the effect of velvetleaf extract on the germination rates of maize, wheat and soybean was investigated. The inhibition rate of velvetleaf water extract on seed germination was dose-dependent and varied among the crop species.
With an increase in the concentration of extracts, a decrease in seed germination in the tested species was observed. As can be seen from Figure 1, crop germination under all concentrations decreased, and the inhibition rate under 10 mg·mL\(^{-1}\) of the water extract was the highest. The velvetleaf extract decreased seed germination to 43.10% for maize, 39.96% for wheat and 8.73% for soybean. Compared with the control, velvetleaf water extracts inhibited maize, wheat and soybean germination. Seed germination in maize and wheat was obviously more sensitive to velvetleaf extract than that in soybean.

![Figure 1.](image-url)  
**Figure 1.** Inhibition rates of velvetleaf extract on seed germination in maize, wheat and soybean after irrigation with velvetleaf water extract at 0 mg·mL\(^{-1}\) (control), 1.25, 2.5, 5 and 10 mg·mL\(^{-1}\). Data are expressed as means ± SE (n = 6).

The water extract of velvetleaf had significant inhibitory effects on the growth of maize, wheat and soybean, and the inhibitory effects were significantly enhanced with an increase in the water extract concentration (Figure 2). Compared with the control, under the treatment with a low concentration of velvetleaf extract, the embryos of the crops became longer and grew well. However, with an increase in the extract concentration, the embryos became shorter and deformed. When the extract concentration reached 10 mg·mL\(^{-1}\), the embryos of maize, wheat and soybean even died. The seed germination of wheat was obviously more sensitive to velvetleaf extract than that of maize and soybean.

Compared with the control, the velvetleaf water extract inhibited the growth of all three crops, as the radicle lengths of the seedlings were reduced under all water extract treatments (Table 2). The results showed that all the water extracts of velvetleaf had an inhibitory effect on the germination and growth of these crops, and the 10 mg·mL\(^{-1}\) water extract had the strongest inhibitory effect. The radicle length of maize, wheat and soybean decreased to 64.98%, 65.10% and 57.35%, respectively, indicating that the germination and growth of wheat were greatly inhibited.

### 3.2. The Effect of Velvetleaf Powder on the Growth and Yield of Three Crops

#### 3.2.1. The Effect of Velvetleaf Powder on the Biomass of Maize, Wheat and Soybean in Pots

The influence of velvetleaf powder on the biomass of the three crops is shown in Table 3. The biomass of the three crops showed a decreasing trend under the application of five different amounts of powder, indicating that the velvetleaf powder could inhibit the growth of the crops. Different amounts of powder had different effects on seedling biomass, with significant differences between plant parts (aboveground and underground).
and among crop species. The belowground dry weight of soybean and maize seedlings was more sensitive than the aboveground dry weight. In contrast, the aboveground dry weight of wheat seedlings was more sensitive than the belowground dry weight.

\[
0 \text{ mg·mL}^{-1} \quad 1.25 \text{ mg·mL}^{-1} \quad 2.5 \text{ mg·mL}^{-1} \quad 5 \text{ mg·mL}^{-1} \quad 10 \text{ mg·mL}^{-1}
\]

Figure 2. Photos of germinated seeds of maize, wheat and soybean treated with velvetleaf water extracts. Concentration of water extracts: 0 mg·mL\(^{-1}\) (control), 1.25, 2.5, 5 and 10 mg·mL\(^{-1}\).

Table 2. Effect of different concentrations of the extracts on the growth parameters of seedlings of maize, wheat and soybean.

| Treatments (mg·mL\(^{-1}\)) | Radicle Length (cm) |
|-----------------------------|---------------------|
|                             | Maize               | Wheat               | Soybean            |
| 0 (CK)                      | 18.96 ± 1.64 a      | 12.32 ± 0.76 a      | 9.38 ± 0.07 a      |
| 1.25                        | 15.33 ± 0.58 b      | 10.69 ± 0.29 b      | 8.19 ± 0.17 b      |
| 2.5                         | 12.62 ± 0.29 c      | 7.26 ± 0.25 c       | 5.81 ± 0.68 c      |
| 5                           | 8.53 ± 0.15 d       | 6.38 ± 0.18 d       | 4.77 ± 0.06 cd     |
| 10                          | 6.64 ± 0.32 e       | 4.30 ± 0.24 e       | 4.00 ± 0.02 d      |

Note: 1.25, 2.5, 5 and 10 are the concentrations of the extract, and 0 represents the control. Data are expressed as means ± SE (n = 6). Within each column, data followed by different lowercase letters are significantly different at \(p < 0.05\).

Table 3. Aboveground and belowground biomass (g plant\(^{-1}\)) of maize, wheat and soybean.

| Crops  | Parts          | Added Amounts of Velvetleaf Powder (mg cm\(^{-3}\)) |
|--------|----------------|---------------------------------------------|
|        |                | 0 (CK) | 1.25 | 2.5 | 5 | 10 |
| Soybean| Aboveground    | 0.85 ± 0.06 a | 0.65 ± 0.02 b | 0.54 ± 0.09 c | 0.46 ± 0.05 d | 0.28 ± 0.08 e |
|        | Belowground    | 0.40 ± 0.02 a | 0.31 ± 0.02 ab | 0.23 ± 0.03 ab | 0.21 ± 0.05 b | 0.12 ± 0.01 c |
| Wheat  | Aboveground    | 0.79 ± 0.08 a | 0.61 ± 0.05 ab | 0.50 ± 0.09 bc | 0.41 ± 0.01 bc | 0.19 ± 0.08 c |
|        | Belowground    | 0.15 ± 0.01 a | 0.13 ± 0.05 ab | 0.11 ± 0.02 ab | 0.08 ± 0.01 c | 0.05 ± 0.02 d |
| Maize  | Aboveground    | 2.85 ± 0.03 a | 2.62 ± 0.09 b | 2.49 ± 0.08 bc | 2.26 ± 0.01 c | 1.87 ± 0.07 d |
|        | Belowground    | 0.70 ± 0.02 a | 0.64 ± 0.05 ab | 0.61 ± 0.03 b | 0.57 ± 0.01 bc | 0.45 ± 0.05 c |

Note: Data are expressed as means ± SE (n = 3). Within each column, data followed by different lowercase letters are significantly different at \(p < 0.05\).
3.2.2. Effects of Added Amounts of Velvetleaf Powder on the Yield and Dry Weight of Maize, Wheat and Soybean in the Field

In order to investigate whether velvetleaf had allelopathic effects on maize, wheat and soybean, we applied different amounts of velvetleaf powder in the field to three kinds of crops. The yield of maize, wheat and soybean under different added amounts of velvetleaf powder (0 kg·m\(^{-2}\), 0.25 kg·m\(^{-2}\), 0.5 kg·m\(^{-2}\), 1 kg·m\(^{-2}\) and 2 kg·m\(^{-2}\)) was tested (Figure 3). The results showed that low added amounts had no obvious inhibitory effect on the three crops, but inhibition showed a dose-dependent effect. That is, the higher the level of velvetleaf powder, the stronger the inhibitory effect on the three crops. The yield per plant of maize, wheat and soybean differed significantly under different added amounts. However, the yield per plant of the three crops showed a decreasing trend. Maize, wheat and soybean were reduced by 52.48%, 15.06% and 43.76% under the 2 kg·m\(^{-2}\) treatment, respectively.

Table 3. Aboveground and belowground biomass (g·plant\(^{-1}\)) of maize, wheat and soybean.

| Crops Parts  | Added Amounts of Velvetleaf Powder (mg·cm\(^{-3}\)) | 0 (CK) | 1.25 | 2.5 | 5 | 10 |
|--------------|-----------------------------------------------|-------|-----|----|---|----|
| Soybean      | Aboveground                                 | 0.85 ± 0.06 | 0.65 ± 0.02 | 0.54 ± 0.09 | 0.46 ± 0.05 | 0.28 ± 0.08 |
|              | Belowground                                 | 0.40 ± 0.02 | 0.31 ± 0.02 | 0.23 ± 0.03 | 0.21 ± 0.05 | 0.12 ± 0.01 |
| Wheat        | Aboveground                                 | 0.79 ± 0.08 | 0.61 ± 0.05 | 0.50 ± 0.09 | 0.41 ± 0.01 | 0.19 ± 0.08 |
|              | Belowground                                 | 0.15 ± 0.01 | 0.13 ± 0.05 | 0.11 ± 0.02 | 0.08 ± 0.01 | 0.05 ± 0.02 |
| Maize        | Aboveground                                 | 2.85 ± 0.03 | 2.62 ± 0.09 | 2.49 ± 0.08 | 2.26 ± 0.01 | 1.87 ± 0.07 |
|              | Belowground                                 | 0.70 ± 0.02 | 0.64 ± 0.05 | 0.61 ± 0.03 | 0.57 ± 0.01 | 0.45 ± 0.05 |

Note: Data are expressed as means ± SE (\(n=3\)). Within each column, data followed by different lowercase letters are significantly different at \(p<0.05\).

Figure 3. Effects of five different added amounts of velvetleaf powder on the yield of maize, wheat and soybean in the field. Data are expressed as means ± SE (\(n=3\)). Columns with different letters indicate significant differences (\(p<0.05\)) among different treatments and the control.

The dry weight of maize, wheat and soybean per plant with different amounts of added velvetleaf powder is shown in Table 4. It can be seen from the table that with an increase in the added amount of velvetleaf powder, plant growth was inhibited, and the dry weight of maize, wheat and soybean per plant decreased under different added amounts of velvetleaf powder. However, the dry weights of three crops were obviously different under the 2 kg·m\(^{-2}\) treatment compared with lower amounts. Compared with the control group, the dry weight per plant of maize, wheat and soybean decreased by 17~51%, 58~79% and 25~54% after 2 kg·m\(^{-2}\) of velvetleaf powder was added.
Table 4. Dry weight (g·plant\(^{-1}\)) of maize, wheat and soybean treated with different added amounts of velvetleaf powder.

| Sampling Stage (Date) | Maize                  | Wheat             | Soybean            |
|-----------------------|------------------------|-------------------|--------------------|
|                       | Treatments (kg Powder of Velvetleaf m\(^{-2}\)) | 0 (CK)  | 0.25  | 0.5   | 1     | 2     |
|                       | Emergence stage (May 5) | 3.43 ± 0.23 a    | 3.27 ± 0.14 a      | 3.01 ± 0.23 ab     | 2.79 ± 0.12 b | 2.34 ± 0.11 b |
|                       | Jointing stage (June 2) | 13.86 ± 0.17 a   | 13.69 ± 0.20 a     | 13.06 ± 0.15 b     | 12.53 ± 0.13 ab | 11.45 ± 0.11 b |
|                       | Tasseling stage (July 5) | 192.4 ± 0.16 a   | 183.27 ± 0.13 a    | 169.01 ± 0.17 b    | 152.79 ± 0.18 b | 95.24 ± 0.11 c |
|                       | Silking stage (August 15) | 265.72 ± 0.09 a | 246.27 ± 0.14 a    | 210.01 ± 0.13 b    | 182.79 ± 0.11 bc | 130.54 ± 0.17 c |
|                       | Maturity stage (September 10) | 384.31 ± 0.13 a | 347.27 ± 0.12 ab   | 300.01 ± 0.11 b    | 279.79 ± 0.06 bc | 212.54 ± 0.12 c |
|                       | Seedling stage (April 20) | 0.91 ± 0.01 a    | 0.82 ± 0.01 a      | 0.66 ± 0.03 b      | 0.51 ± 0.02 b    | 0.33 ± 0.01 c   |
|                       | Jointing stage (May 20)  | 1.80 ± 0.03 a    | 1.09 ± 0.01 ab     | 0.78 ± 0.02 b      | 0.61 ± 0.02 bc   | 0.42 ± 0.03 c   |
|                       | Heading stage (June 15)  | 2.71 ± 0.02 a    | 1.82 ± 0.03 ab     | 1.36 ± 0.01 b      | 0.99 ± 0.03 bc   | 0.57 ± 0.01 c   |
|                       | Filling stage (July 25)  | 3.50 ± 0.03 a    | 3.22 ± 0.02 a      | 2.66 ± 0.01 b      | 2.01 ± 0.02 bc   | 1.43 ± 0.03 c   |
|                       | Maturity stage (August 10) | 4.03 ± 0.01 a   | 3.82 ± 0.03 a      | 3.46 ± 0.01 b      | 2.81 ± 0.03 bc   | 1.65 ± 0.02 c   |
|                       | Germination stage (May 5) | 1.02 ± 0.21 a    | 0.91 ± 0.13 a      | 0.73 ± 0.11 b      | 0.62 ± 0.20 bc   | 0.47 ± 0.14 c   |
|                       | Branching stage (June 2) | 6.88 ± 0.16 a    | 6.69 ± 0.21 a      | 6.47 ± 0.12 a      | 6.02 ± 0.09 a    | 4.77 ± 0.13 b   |
|                       | Flowering stage (July 5) | 9.36 ± 0.11 a    | 9.09 ± 0.12 a      | 8.63 ± 0.20 b      | 8.01 ± 0.18 b    | 6.47 ± 0.13 c   |
|                       | Bulking stage (August 15) | 14.41 ± 0.18 a  | 13.91 ± 0.13 a     | 13.03 ± 0.12 ab    | 12.22 ± 0.21 b   | 10.47 ± 0.16 c  |
|                       | Maturity stage (September 10) | 27.32 ± 0.12 a | 26.91 ± 0.15 a     | 26.33 ± 0.13 a     | 25.62 ± 0.09 b   | 20.47 ± 0.17 c  |

Note: Data are expressed as means ± SE (n = 3). Within each column, data followed by different lowercase letters are significantly different at p < 0.05.

3.3. Effects of Velvetleaf Powder on Lipid Peroxidation and Antioxidant Activity in Pots

The content of MDA and the activity of POD, SOD in maize, wheat and soybean treated with of velvetleaf powder added at different amounts were measured. It was found that MDA content increased gradually with an increase in the added amount (1.25, 2.5, 5 and 10 mg·cm\(^{-3}\)). The lowest MDA content was found in control crops; however, the MDA content was dose-dependent in the treated groups. When the maximum added amount was 10 mg·cm\(^{-3}\), the MDA content of maize, wheat and soybean was 2.87, 2.46 and 1.54 times that of the control group, respectively. Increased MDA content suggested the allelopathic inhibition effects of velvetleaf in maize, wheat and soybean (Figure 4A).

Maximum SOD activity was measured in crops treated with lower added amounts of velvetleaf powder, while the highest added amounts of velvetleaf powder (10 mg·cm\(^{-3}\)) significantly suppressed the SOD activities of maize, wheat and soybean. The decreased enzyme activity of SOD at 10 mg·cm\(^{-3}\) velvetleaf powder indicated that the SOD antioxidant defense system failed (Figure 4B).

Similarly, maximum POD activity was measured in maize and wheat treated with lower amounts of velvetleaf powder, while the highest added amounts of velvetleaf powder (10 mg·cm\(^{-3}\)) significantly suppressed the POD activity of maize and wheat. The decreased enzyme activity of POD at 10 mg·cm\(^{-3}\) indicated that the antioxidant defense system failed. On the contrary, a gradual increase in POD activity was measured in soybeans treated with increased amounts of velvetleaf powder (1.25, 2.5, 5 and 10 mg·cm\(^{-3}\)). The lowest POD activity was found in the control; however, under the treatments, POD activity was
dose-dependent. Increased POD activity indicated that POD antioxidant defense played a crucial role in soybean (Figure 4C).

Figure 4. The (A) MDA contents, and (B) SOD and (C) POD activities of maize, wheat and soybean in response to 0 (control), 1.25, 2.5, 5 and 10 mg·cm$^{-3}$ of added velvetleaf powder. Data are presented as means ± SE ($n = 3$).

3.4. Effect of Velvetleaf Powder or Extract on Root Structure and Physiological Indices

3.4.1. Effects of Velvetleaf Powder on Root Physiological Indices in Pots

Velvetleaf powder had a varying effect on the content of sugar and protein. The allelopathic stress sharply reduced the protein and sugar content at the highest (10 mg·cm$^{-3}$) added amount of velvetleaf powder. The protein and sugar content reduced to 20.37 and 1.65 mg·g$^{-1}$·FW for maize, 13.77 and 0.06 mg·g$^{-1}$·FW for wheat, and 16.75 and 1.03 mg·g$^{-1}$·FW for soybean at the highest amounts of powder (Figure 5A,B).

Maximum root system activity was measured in maize, wheat and soybean treated with lower added amounts of powder, while the highest added amount of velvetleaf powder (10 mg·cm$^{-3}$) significantly suppressed the root system activity of maize, wheat and soybean. The decrease in root system activity under 10 mg·cm$^{-3}$ of velvetleaf powder suggested that the roots of the crops were severely damaged. The root system activity under 10 mg·cm$^{-3}$ decreased to 6.70 mg·g$^{-1}$·h$^{-1}$ in maize, 54.20 mg·g$^{-1}$·h$^{-1}$ in wheat and 39.46 mg·g$^{-1}$·h$^{-1}$ in soybean (Figure 5C).

3.4.2. Effect of Velvetleaf Extract on Root Tip Structure in Petri Dishes

Fluorescence microscopy was used to study the structure of the root tip. As shown in Figure 6, for the control group, the cross-section was relatively regular and round, the tissues of each part are complete and closely arranged with each other, and the cell shape is full and regular. On the contrary, the cell morphology of the maize, wheat and soybean treated with the water extract of velvetleaf showed distortion and structural damage in the transverse section of the root tip, and the normal circular shape was no longer found. There were faults between the tissues, holes appeared in the cells, and the root vascular bundles were scattered loosely around the central pulp. When the concentration of the velvetleaf extract increased to 10 mg·mL$^{-1}$, the root tip of the three crops suffered more serious damage, becoming dehydrated and wizened, with a loss of tissue structure and characteristics, large tissue faults between the cells, a loss of continuity, and cells showing
cytoplasmic vacuolation. The number of vascular bundles in maize and wheat reduced; however, the cells of soybean were severely shriveled, and furrowed cavities appeared in the tissues.

Figure 5. Root system activity and content of sugar and protein in (A) maize, (B) wheat and (C) soybean after the addition of 0 (control), 1.25, 2.5, 5 and 10 mg cm$^{-3}$ of velvetleaf powder. Data are expressed as means ± SE ($n = 3$). Columns with different letters indicate significant differences ($p < 0.05$) among different treatments and the control.

4. Discussion

Analyses of seed germination and seedling growth in recipient plants treated with donor plants have become one of the main methods of allelopathic study. The germination status of seeds directly determines the number and proportion of individuals in the natural community, and the growth status of seedlings after germination directly determines...
whether they can grow normally and achieve survival status in the community [34]. In this study, the water extract of velvetleaf had a significant allelopathic effect on the seed germination and seedling growth of maize, wheat and soybean, and the allelopathic inhibition increased with an increase in the extraction concentration (Figures 1 and 2). In addition, Ben et al. [35] found that the inhibition rate of crude extracts of *Eucalyptus erythrocorys* L. on wheat seed germination and seedling growth increased with increasing extract concentration. Compared with seed germination, seedling growth, especially radicle elongation, was more sensitive to the effect of the extract. In our petri dish experiment, when the concentration of velvetleaf extract was 10 mg·mL\(^{-1}\), compared with the control group, the germination rates of maize, wheat and soybean were 43.10%, 39.96% and 8.73%, and the radicle lengths were reduced to 64.98%, 65.10% and 57.35%, respectively (Table 2). These results indicated that effect of the extract on growth of three crops seedlings had a more significant inhibitory effect than it did on seed germination.

As an important index of seedling growth, plant biomass is of great significance for the study of allelopathy. In general, the growth status and environmental conditions of cultivated crops (temperature, soil moisture, nitrogen and phosphorus fertilizer application, light, etc.) have different effects on the belowground and aboveground parts of crops. Šćepanović et al. [36] showed that extracts of several cover crops (CCs) with different concentrations had significant effects on *Ambrosia artemisiifolia* L. The allelopathic effects on radicle length, seedling fresh weight and other early growth indicators differed depending on the species and concentration. In our pot experiment, different amounts of velvetleaf powder had different effects on crop biomass, but overall, the effect was dose-dependent. When the content of velvetleaf powder was 10 mg·cm\(^{-3}\), the belowground dry weight of soybean and maize seedlings decreased by 70% and 35.71%, respectively, and these parts were more sensitive than the aboveground parts. In contrast, the dry weight of wheat seedlings decreased by 75.95% in the aboveground parts, which were more sensitive than the belowground parts (Table 3). Shah et al. [37] conducted a two-year field experiment of *Prosopis juliflora* (Swartz) DC. on weed weed control and yield, and found that water extract of *Prosopis juliflora* (Swartz) DC. may contain a variety of phytotoxic substances, which significantly reduced weed density, fresh weed biomass, dry weed biomass, wheat yield and biomass after application. This may be due to the adverse effects of various phytotoxic substances in the water extract on wheat growth and yield. In our field experiment, compared with the control group, under the treatment with 2 kg·m\(^{-2}\) velvetleaf powder, the yield per plant of maize, wheat and soybean decreased from 318.04 g, 6.17 g and 21.15 g to 270.14 g, 3.47 g and 10.05 g, respectively. Among these, maize yield per plant decreased by the most significant percentage (Figure 3). The dry weight per plant of wheat decreased by 58–79% during the five sampling stages, with the largest decrease (Table 4).

A common consequence of allelopathy is oxidative damage [38], which is caused by the production of reactive oxygen species (ROS) in plant cells [39], and ROS can block plant metabolism [40]. In this study, with the addition of different doses of velvetleaf powder, the MDA content of three crops continued to increase, and the MDA content of maize even increased to 2.87 times that of the control group (Figure 4A). This may be be because the crop cell membranes had been severely damaged. In addition, when plants are exposed to allelopathic substances, this will immediately trigger the activity of antioxidant enzymes such as POD and SOD to remove reactive oxygen species, mitigate the effects of oxidative stress and carry out self-protection in plants [41]. In our experiments, SOD and POD activities in maize and wheat first increased with an increase in the added amount of velvetleaf powder, and then these activities decreased (Figure 4B,C). Similar results of SOD activity were also observed in soybean. The decrease in SOD and POD activity indicated that with the addition of higher amounts of powder, the scavenging function of the antioxidant enzymes failed [42], thus leading to cell damage. No turning point was found in the POD activity of soybean among the treatments; this may be because the antioxidant defense in soybean worked even under the highest amounts of powder.
Soluble sugars and proteins participate in osmotic regulation, which are important nutrients and regulatory factors for plant growth and development, and play an important regulatory role in plants' physiological metabolism. Sugar and protein contents in seedlings affect the growth, root length and fresh weight of seedlings [43], and thus affect plant growth and development. Hussain et al. [44] showed that the exposure of plants to allelochemicals resulted in protein denaturation and significantly reduced protein synthesis, resulting in significantly reduced protein content in leaves. Peng [45] found that a low concentration of water extract of maize leaves significantly promoted the germination and seedling growth (root length, stem height and fresh weight) of three medicinal plants, but with an increase in the water extract concentration, the stimulating effect gradually weakened and even became an inhibitory effect. In our experiment, the higher level of velvetleaf powder significantly reduced the content of sugar and protein (Figure 5A,B), and the root activity also decreased (Figure 5C). In addition, velvetleaf had a dose-dependent inhibitory effect on the content of sugar and protein and the root activity of the three crops. Researchers [46] treated wheat seedlings using a culture medium of *Solidago canadensis* L., which has allelopathic potential, resulting in severely blurred root crowns, complete ablation, degradation of cells in the meristem zone, and distortion of the conduit and surrounding cells in the mature zone. Romero et al. [47] found that a water extract of *S. deppei* inhibited the growth of the meristem and elongation zone of tomato (*Lycopersicon esculentum* Miller) seedlings. Yang et al. [48] found that upland rice seedlings treated with *Eupatorium adenophora* Spreng. leached two main allelopathic substances resulted in short, swollen roots and lateral root deficiency. In our experiment, under treatment with velvetleaf extract, the anatomical structure of the root tip changed obviously, which mainly manifested as the large intercellular space of the root cap, meristem and stationary center. The root tip cells in the cross-section in the control group were closely arranged with a regular structure, but these were disordered in the treated group, and the central pulp was depressed, which made the root system unable to absorb water and nutrients normally, and the growth was seriously disturbed and inhibited (Figure 6). This shows that the velvetleaf extract can destroy and damage the root tip cells of maize, wheat and soybean, leading to cell death and inhibiting their activities [49].

5. Conclusions

In this study, the allelopathic effects of velvetleaf powder and extract on maize, wheat and soybean were confirmed by field experiments, petri dish experiments and pot experiments. In addition, velvetleaf powder and extract were found to damage the root tip cells of maize, wheat and soybean, reduce soluble sugar and protein content, and inhibit root activity, thereby affecting the seed germination, seedling growth, yield and biomass of the three crops. Therefore, we concluded that velvetleaf may have allelopathic effects on the three crops by changing the antioxidant enzyme activity, root activity and root tip structure. However, the mechanisms of allelopathy are often complex, and other studies are needed to understand the mechanisms by which the allelopathic effect is exerted. Further studies are underway to paint a comprehensive picture of the toxic nature of root residues and to analyze other potential allelochemicals and their effect on crop growth.

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