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Assessment of the Allelopathic Activity of Various Parts of Platycodon (*Platycodon grandiflorus*) and Its Mitigation by Activated Carbon

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Abstract: Platycodon is a medicinal plant of considerable pharmacological and dietary value. With the growing demand, agricultural production is increasing. However, the continuous cropping significantly causes negative impacts on its yield and quality. In this study, in order to solve the problem of continuous cropping, we evaluated the allelopathic activity of Platycodon and investigated the potential use of activated carbon for mitigating the negative impacts of allelopathic chemicals produced by Platycodon. The sandwich method (method for assaying the allelopathic activity of each part of a plant) was employed to evaluate the allelopathic activity of different parts (leaves, stems, and roots) of Platycodon. The inhibitory effects of various Platycodon plant parts were assessed based on their effects on lettuce seedling growth. At a concentration of 10 mg parts/10 mL agar, the average inhibition rates of Platycodon leaves on the radicle and hypocotyl growth of lettuce were 79.4% and 61.8%, stems 58.0% and 45.7%, and roots 53.4% and 49.3%, respectively. At a concentration of 50 mg parts/10 mL agar, the inhibitory effects were as follows: leaves (91.9%, 72.2%), stems (79.5%, 60.3%), and roots (71.4%, 65.2%). The effect of activated carbon on the adsorption of allelopathic substances was investigated, and the results of the sandwich method with a concentration of 10 mg parts/10 mL agar showed the following growth-inhibitory effects on lettuce seedlings and hypocotyls—roots (27.8%, 25.7%), leaves (13.3%, 25.7%), and stems (9.1%, 13.6%)—in each case showing a significant decrease in the inhibitory activity. The plant box method (method for assaying the allelopathic activity of plant root exudates) was employed to evaluate the activity of Platycodon root exudate. The growth inhibition rates of lettuce radicle and hypocotyls were 45.5% and 18.9%, respectively. The plant box method with addition of activated carbon revealed average rates of promotion of 16.7% and 4.7% on the growth of lettuce seedlings and hypocotyls, respectively. The results of this study demonstrated that activated carbon has a mitigating effect on allelopathic inhibition associated with the different plant parts and root exudation of Platycodon and provide a potential solution for overcoming problems associated with the continuous cropping of Platycodon.

Keywords: activated carbon; Platycodon; continuous cropping problem; exudation of allelochemicals

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1. Introduction

Platycodon (*Platycodon grandifloras* (Jacq.) A. DC.), commonly known as balloon flower, is a perennial herb belonging to the family Campanulaceae. It is also referred to as *ling-danghua* in Chinese and is known as *ulundanchagan* in Mongolian. The roots of this plant are extensively used in traditional Chinese medicine. Medicinal values of Platycodon are hypotension, lipid reduction, atherosclerosis, inflammation, relieving cough and phlegm, promoting cholic acid secretion, and as an antioxidant. It is estimated that the annual production value is around $7.27 billion. Its aboveground parts are consumed as a vegetable. It is primarily distributed across various provinces in northeast and central China, as well as Guangdong, northern Guangxi, Guizhou, southeastern Yunnan (Mengzi, Yanshan, Wenshan), Sichuan (east of Pingwu, Liangshan), Shaanxi, and other regions. It is also found in parts of South Korea, Japan, and southern regions of the Russian Far East and East Siberia. Platycodon typically thrives in sunny grasslands and shrublands at elevations below 2000 meters and is less commonly found in forest understories [1]. Due to its high medicinal and food value, the market demand for Platycodon is increasing, and artificial cultivation has become the main way to supply the market.

Medical research and analysis show that Platycodon contains a variety of active chemical components, mostly comprising Platycodon total saponins [2]. In addition, Platycodon also contains polysaccharides, flavonoids, amino acids, fatty oils, fatty acids, inorganic elements, vitamins, volatile oils [3], and a large amount of unsaturated fatty acids. Therefore, Platycodon has high medicinal and edible value. Studies have shown that Platycodon saponin has weight-loss activity [4]. Platycodon also has the effects of reducing tobacco toxicity and controlling the increase in blood alcohol content in the human body [5]. Therefore, Platycodon can also be processed into thin strips to make cold dishes, and some can also be made into pickles, cans, and other products. It is a very popular functional healthy food [6]. The demand for medicinal and healthy food production of Platycodon has increased, and Platycodon has become an important crop in agricultural production. At the same time, it will bring greater economic benefits to the development of Platycodon planting areas. Therefore, research on Platycodon has attracted widespread attention.

The limited availability of land resources suitable for cultivating authentic medicinal herbs is influenced by factors such as soil conditions and planting restrictions. Continuous cropping or replanting is common in this context [7]. Crop rotation is a beneficial measure to overcome the problems of continuous cropping; however, the intercropping duration for medicinal plants is often lengthy. For instance, *Scrophularia ningpoensis* requires an interval of 3–4 years before it can be planted again in the same soil. Ginseng and American ginseng may need more than 30 years before being replanted at the same location [8]. Intercropping with *Salvia miltiorrhiza* can take as long as 8 years [9]. These extended intercropping intervals significantly impact the stability of the supply of traditional Chinese medicinal herbs. Therefore, finding methods to alleviate the challenges of continuous cropping is crucial for the sustainable development of the traditional Chinese medicine industry.

However, continuous Platycodon cropping seriously hampers the sustainable development of its cultivation. Previous studies have shown that the continuous cultivation of Platycodon for 2 years results in significantly decreased yield and quality, while continuous cultivation for 4–5 years may lead to severe root rot, causing a reduction in yield of over 50% or even complete crop failure [10]. Despite attempts to address obstacles to continuous cropping through pest control, disease management, and cultivation techniques, the results remain unsatisfactory [11]. Moreover, the allelopathic effects of Platycodon leaves have been characterized, revealing their strong allelopathic inhibitory effect on the growth of lettuce seedlings and hypocotyls of test plants [12]. Considering the decline in emergence rates and growth damage observed in replanted crops, it is reasonable to speculate that Platycodon may induce autotoxicity through the release of allelopathic substances.

Allelopathy is a phenomenon in which plants influence the growth of surrounding plants (or microorganisms) by releasing natural compounds, resulting in inhibitory or stimulatory effects [13]. This phenomenon was first named “allelopathy” by German

botanist Hans Molisch in 1937, and the compounds causing allelopathy are referred to as allelochemicals. Allelochemicals are primarily released via three pathways: volatilization from the aboveground parts of plants, leaching or shedding from plant debris, and root exudation. They affect not only the growth of neighboring plants but also may impact the plant's community or its growth [14].

Continuous cropping poses a significant challenge in the cultivation of medicinal herbs, and over 40% of medicinal herbs are artificially cultivated in China. Among the cultivated varieties, root-based medicinal herbs make up approximately 70%, and obstacles to continuous cropping are prevalent in their cultivation processes [15,16]. Medicinal herbs such as *Rehmannia glutinosa*, *Pinellia ternata*, *Pseudostellaria heterophylla*, *Panax ginseng*, *Lilium brownii*, *Panax pseudoginseng*, *Panax quinquefolius*, and *Salvia miltiorrhiza* all face issues related to continuous cropping obstacles [17–24]. The phrase "continuous cropping obstacles" refers to the phenomenon whereby consecutive planting in the same plot of land results in a decline in seedling emergence and growth inhibition [25]. The main causes include an increase in soilborne pathogens or exacerbation by pests and diseases [26], impaired absorption of soil nutrients, deterioration of soil physicochemical properties, and allelopathy [27,28]. Autotoxicity caused by allelopathic substances released by plants is a crucial factor contributing to the formation of obstacles to continuous cropping. Numerous studies have shown a close correlation between the health of medicinal plants under continuous cropping conditions and the content of root exudates, especially by observing the accumulation of organic acids in continuously cropped soil. These organic acids, phenolic acids, terpenoids, coumarins, flavonoids, and other compounds have been identified as allelopathic substances [29]. Some root exudates induce the production of reactive oxygen species, disrupt root tip cells, and affect the levels of chlorophyll and osmoregulatory substances, thus inhibiting the growth of roots and shoots. The allelopathic inhibitory feedback exhibited by roots is more pronounced than that of stems and leaves, which may be a primary mechanism through which allelopathic substances influence the content of active components, especially underground parts, in medicinal plants [30]. Since Platycodon is a type of root-based medicinal herb, the allelopathic effects of root exudates are crucial elements that need to be measured and analyzed when examining the causes of obstacles to continuous cropping.

The allelopathic activity of the entire Platycodon plant and its various parts concerning its seed germination and seedling growth have been investigated, and strong allelopathic inhibitory effects were observed [12]. This suggests that the continuous cropping obstacles associated with Platycodon may arise from autotoxicity caused by allelochemicals released by the plant itself [31]. During the process of continuous cropping problems, root exudates are also one of the important influencing factors. Therefore, studying the impact of root exudates on plant growth is a key aspect in elucidating the reasons behind continuous cropping obstacles [32]. However, there is currently more research on the impact of aboveground allelochemicals on seedling growth and germination, while studies on the allelopathic effects of the root exudates of Platycodon are relatively scarce. In this study, the allelopathic effects of Platycodon roots and their exudates were initially measured to explore the potential role of the roots in the formation of obstacles to continuous cropping. Subsequently, the allelopathic effects of Platycodon root exudates were comprehensively assessed using the plant box method to better infer the impacts resulting from their contribution to continuous cropping problems. Finally, to understand the allelopathic effects of the various parts and root exudates of Platycodon, activated carbon, a material effective in controlling allelopathic inhibition in crops such as asparagus and peach trees, was introduced to test its effects on the absorption of Platycodon self-released allelopathic substances.

The definition of activated carbon is not precise, and it is generally described as a substance with a large surface area and strong adsorption capacity that is mainly composed of carbon [33]. Activated carbon has various applications, such as serving as a deodorant in refrigerators and an adsorbent in industries. Its raw materials mainly include wood charcoal, coconut shells, and brown coal. In the agricultural field, the use of activated

carbon has been studied for several types of plants, and some have even been put into practical use in Japan. For example, although there are studies on the role of activated carbon in alleviating continuous cropping problems in crops such as cucumber, beans, sugar beets, and asparagus [34–37], research on such problems related to Platycodon is relatively limited.

Therefore, this research investigated the absorptive effects of activated carbon on the allelopathic effects of the various parts and root exudates of Platycodon toward establishing a scientific basis for the study of materials that alleviate obstacles to continuous cropping. This study aims to demonstrate the role of potential autotoxicity of Platycodon from the perspective of allelopathy and to investigate the efficacy of activated carbon agents in alleviating continuous cropping obstacles in Chinese Platycodon cultivation.

2. Materials and Methods

2.1. Sample Cultivation and Collection

Platycodon seeds (REF-Platycodon: Gansu Ruierfeng Agricultural Science and Trade Co., Ltd., Lanzhou, China) were cultivated in experimental greenhouses from 20 May to 20 October 2023 at Meiji University Faculty of Agriculture. The seeds were planted in culture cups (7.5 cm in diameter) using vegetable cultivation soil (Sakata Seed Co., Ltd., Yokohama, Japan, Super Mix A 40 L). Liquid fertilizer solution (Hyponex®(N-P-K = 6-10-5), Hyponex Japan Corp., Ltd. Osaka, Japan) diluted 1000 times was applied every day for one week after planting. The leaf, root, and stem samples were collected at 60 days (20 July), 90 days (20 August), and 150 days (20 October) of cultivation. After each sampling, the samples were dried in a 60-degree oven for 48 h. The plant box method involved selecting whole plants that weighed approximately 100–300 mg for testing.

2.2. Experimental Methods

Sandwich method (Figure 1): This method was employed to determine the allelopathic effects of the different plant components. A 6-well cell culture plate (Multidish 6 Wells, Nunclon Delta Si, Thermo Fisher Scientific, Shanghai, China) was used, and the prepared samples were divided into two amounts: 10 mg and 50 mg (the reason for choosing 10 mg parts/10 mL agar and 50 mg parts/10 mL agar was the number of plants present at the site) [38], placed in 3 wells each. The samples were arranged in a sandwich-like layer, with lettuce (*Lactuca sativa*) seeds (lettuce seed varieties: Cisco, sold by Takii Seed Co., Ltd., Kyoto, Japan) planted on top. After 3 days of emergence, the hypocotyl and radicle lengths of the seedlings were measured to evaluate the biological activity of the samples [38].

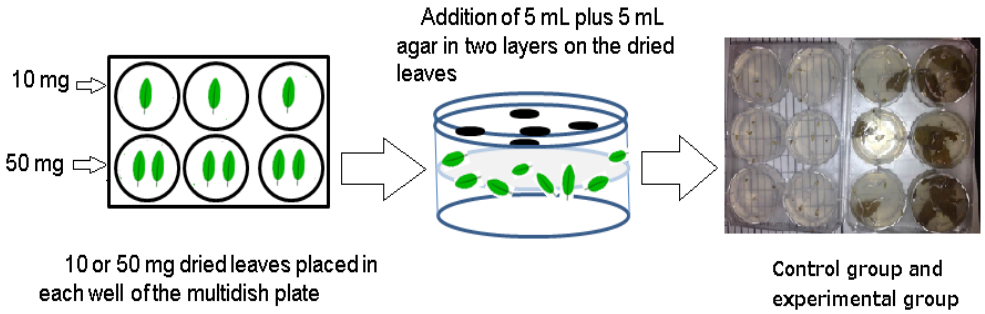


Figure 1. Schematic diagram of the sandwich method.

Plant box method (Figure 2): Plants that had been growing for 2–3 months in culture cups, with fresh weights ranging approximately 50–600 mg, were selected for this experiment. They were transplanted into test boxes containing 0.75% sterilized agar after washing the soil off the roots. After 5 days, the root growth of the lettuce seedlings was measured to assess the allelopathic effects of the plant samples [39].

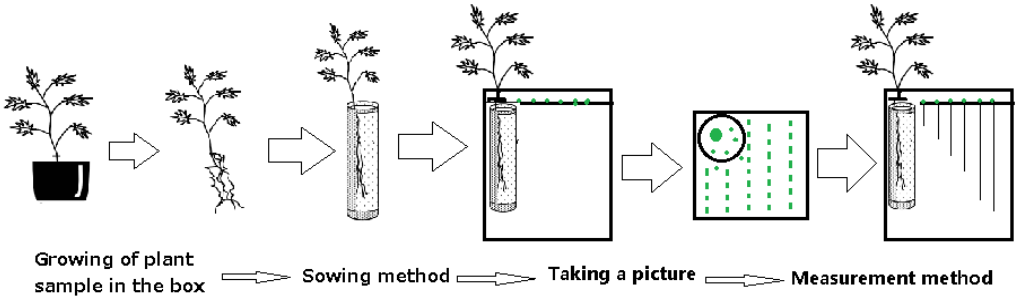


Figure 2. Schematic diagram of the plant box method.

Adsorption test using activated carbon (Figure 3): Activated carbon has a good ability to adsorb allelopathic substances based on previous studies. A flowable activated carbon agent (Otsuka Chemical Co., Ltd., Osaka, Japan) was added to the agar medium used in the sandwich and plant box methods at a dilution of 25 times (1:25). The results with and without activated carbon were compared [40].

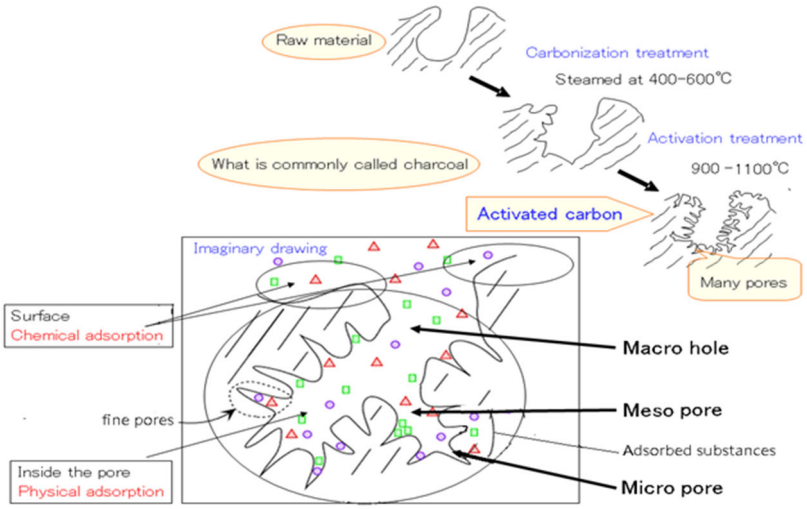


Figure 3. Model of activated carbon-adsorbing substances. ○, □, △ Each is a growth inhibitory substance (allelopathic substance).

2.3. Statistical Analysis

All experiments were conducted in triplicate at least, and the statistical analysis software used was Microsoft Excel 2010 version and IBM SPSS, 29.0.2.0 Statistics software.

3. Results

3.1. Bioassay Tests for Assessing the Allelopathic Activity of Platycodon Parts at Different Ages

First assessment (after 60 days): The results revealed that among the various parts of Platycodon, the leaves exhibited the strongest inhibitory effect upon the first assessment. At a concentration of 10 mg parts/10 mL agar, the inhibition rates of the lettuce radicle and hypocotyls were 73.9% and 56.2%, respectively. The next-highest inhibitory effect was shown by the root (41.2%, 43.1%) and the lowest by the stem (22.7%, 5.4%), respectively. At a concentration of 50 mg parts/10 mL agar, the leaves also caused the highest reduction in radicle and hypocotyls (88.1%, 61.1%), roots (70.9%, 52.6%), and stems (77.2%, 54.2%), respectively.

Second assessment (after 90 days): At a concentration of 10 mg parts/10 mL agar, the inhibition percentages, from the highest to the lowest, were as follows: leaves (79.8%, 65.0%), roots (42.6%, 35.9%), and stems (46.8%, 32.4%) in radicle and hypocotyls, respectively. At 50 mg parts/10 mL agar, the leaves (92.4%, 34.9%), stems (77.5%, 60.0%), and roots (57.4%, 40.0%) continued to display inhibitory effects. Additionally, during the flowering season of

Platycodon, the flower part also demonstrated inhibitory effects, with inhibition rates of 67.4% and 49.0% at 10 mg/10 mL and 81.2% and 59.7% at 50 mg parts/10 mL agar. Third assessment (after 150 days): At a concentration of 10 mg parts/10 mL agar, the inhibition percentages in radicle and hypocotyls, respectively, were as follows: leaves (84.4%, 64.2%), roots (76.4%, 68.9%), and stems (69.5%, 54.8%). At 50 mg parts/10 mL agar, the leaves caused 95.4% and 86.9%, roots 92.3% and 82.6%, and stems 30.5% and 45.3%. Overall, according to the three assessments, at a concentration of 10 mg parts/10 mL agar, the average inhibition rates for the leaves and roots were 79.4% and 53.4%, respectively, and at a concentration of 50 mg parts/10 mL agar, the average inhibition rates for the leaves and stem were 91.9% and 78.3%, respectively (Table 1).

Table 1. Allelopathic activity of different Platycodon components according to the sandwich method.

		10 mg			50 mg		
Date	**Part**	**R**	**H**	**G**	**R**	**H**	**G**
20 July	Leaf	26.1 ± 1.3	43.8 ± 1.4	100 ± 0.0	11.9 ± 1.0	38.9 ± 1.7	86.7 ± 11.5
	Root	58.8 ± 0.7	56.9 ± 0.3	100 ± 0.0	29.1 ± 0.5	47.4 ± 0.3	100 ± 0.0
	Stem	77.3 ± 2.2	94.6 ± 2.5	100 ± 0.0	22.8 ± 1.3	45.8 ± 1.4	93.3 ± 8.9
20 August	Leaf	20.2 ± 1.3	34.9 ± 1.6	93.3 ± 8.9	7.6 ± 1.4	31.4 ± 2.3	86.7 ± 10.9
	Root	57.4 ± 1.4	64.1 ± 1.1	100 ± 0.0	48.9 ± 0.7	60.0 ± 0.6	100 ± 0.0
	Stem	53.1 ± 1.7	67.6 ± 1.9	100 ± 0.0	22.5 ± 1.5	40.0 ± 2.0	100 ± 0.0
20 October	Leaf	15.6 ± 1.6	35.8 ± 2.1	100 ± 0.0	4.6 ± 0.5	13.1 ± 0.7	93.3 ± 8.9
	Root	23.6 ± 3.5	31.0 ± 2.6	93.3 ± 8.9	7.7 ± 0.7	17.0 ± 0.9	100 ± 0.0
	Stem	30.5 ± 3.4	45.2 ± 3.1	100 ± 0.0	19.9 ± 2.5	33.1 ± 2.5	100 ± 0.0
AIR	Leaf	79.4	61.8	0	91.9	72.2	6.7
	Root	53.4	49.3	2.2	71.4	58.5	4.4
	Stem	46.3	30.8	2.2	78.3	60.4	2.2

Values are the % of growth compared to the control; R: radicle; H: hypocotyl; AIR: average inhibition rate; G: germination ratio (%).

These results indicate that various parts of Platycodon at different ages possess a significant allelopathic inhibitory effect on the growth and germination of lettuce seedlings. The greatest inhibitory effect was caused by the leaves at different ages. The inhibition rates increased as the concentration of Platycodon residues increased.

3.2. Effect of Activated Carbon on the Adsorption of Allelopathic Substances from Various Platycodon Components

In this experiment, the activated carbon mixed with agar media at a ratio (1:25) was utilized as part of the sandwich method to investigate its ability to reduce the negative effect of allelochemicals. The results of the first test (after 90 days) showed inhibitory effects on the growth of lettuce seedling roots and hypocotyls at a concentration of 10 mg parts/10 mL agar in the following order: roots (35.7%, 35.1%), leaves (9.7%, 19.1%), and stems (−18.9%, −6.2%). For the stem test, the growth rate of the test plants exceeded that of the control group; thus, it is represented with negative values for statistical purposes. At a concentration of 50 mg parts/10 mL agar, inhibitory effects on the growth of lettuce seedlings roots and hypocotyls were observed in the following order: leaves (45.5%, 37.4%), stems (31.5%, 28.5%), and roots (24.5%, 27.2%), respectively.

In the second test (after 150 days), at a concentration of 10 mg parts/10 mL agar, the inhibitory effects on the growth of lettuce seedling roots and hypocotyls were observed in the following order: stems (37.1%, 33.5%), roots (19.9%, 16.6%), and leaves (16.8%, 32.3%). On the other hand, at a concentration of 50 mg parts/10 mL agar, the inhibitory effects on growth were observed in the following order: roots (61.6%, 49.0%), leaves (56.4%, 54.2%), and stems (28.0%, 34.7%) for radicles and hypocotyls, respectively. The stems' germination rate was 92.3% at a concentration of 50 mg parts/10 mL agar, while that of all other tested areas was 100%.

The average results of the two tests showed that at a concentration of 10 mg parts/10 mL agar, the average inhibition rates were 13.3% for the leaves, 27.8% for the roots, and 9.1% for the stems. At a concentration of 50 mg parts/10 mL agar, the average inhibition rates were 50.9% for the leaves, 43.0% for the roots, and 29.7% for the stems. Overall, the addition of the activated carbon reagent reduced the allelopathic inhibition rates by various parts of Platycodon (Figures 4–6).

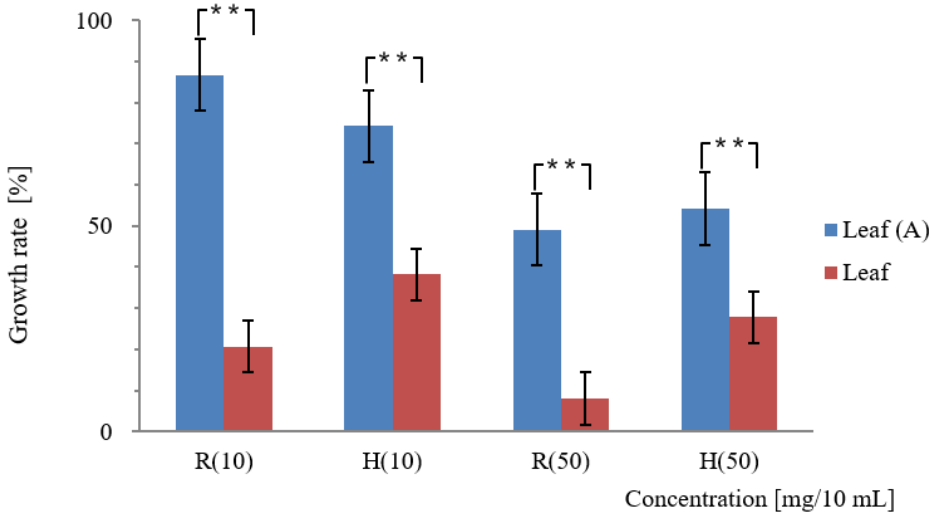


Figure 4. Sandwich method for the evaluation of allelopathic effects of Platycodon leaves. "A" indicates the results of the trial with activated carbon; results lacking identification represent the trial without the addition of activated carbon. At a concentration of 10 mg parts/10 mL agar, the difference between the results of the non-added and added activated carbon test areas was 2.1%, and this was a significant difference (<5.0%). Bars indicate standard deviation, ** indicates a significant difference at the 0.1% level by *t*-test.

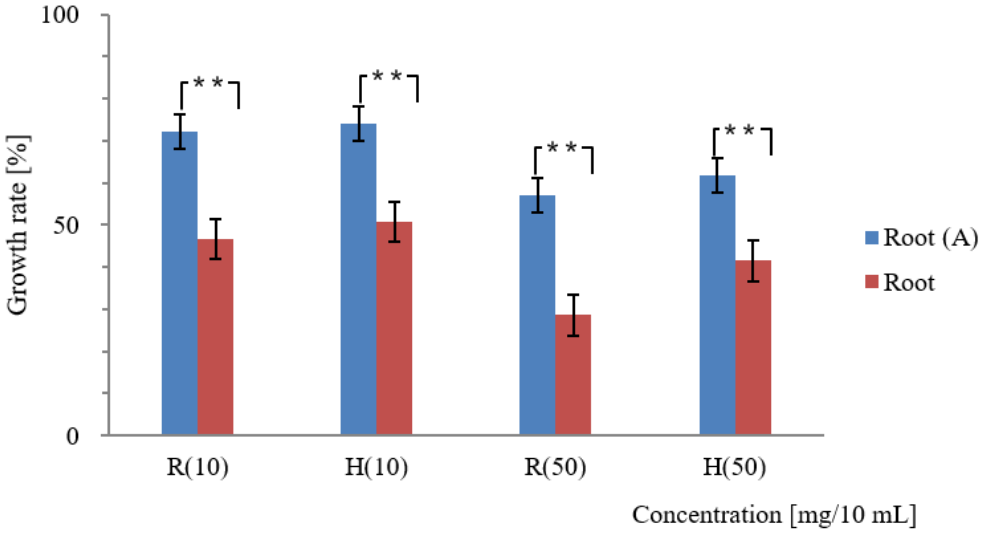


Figure 5. Sandwich method for the determination of the allelopathic effects of Platycodon roots. "A" indicates the results of the trial with activated carbon; results lacking identification represent the trial without the addition of activated carbon. At a concentration of 10 mg parts/10 mL agar, the difference between the results of the non-added and added activated carbon test areas was 0.4%, and this was a significant difference (<5.0%). Bars indicate standard deviation, ** indicates a significant difference at the 0.1% level by *t*-test.

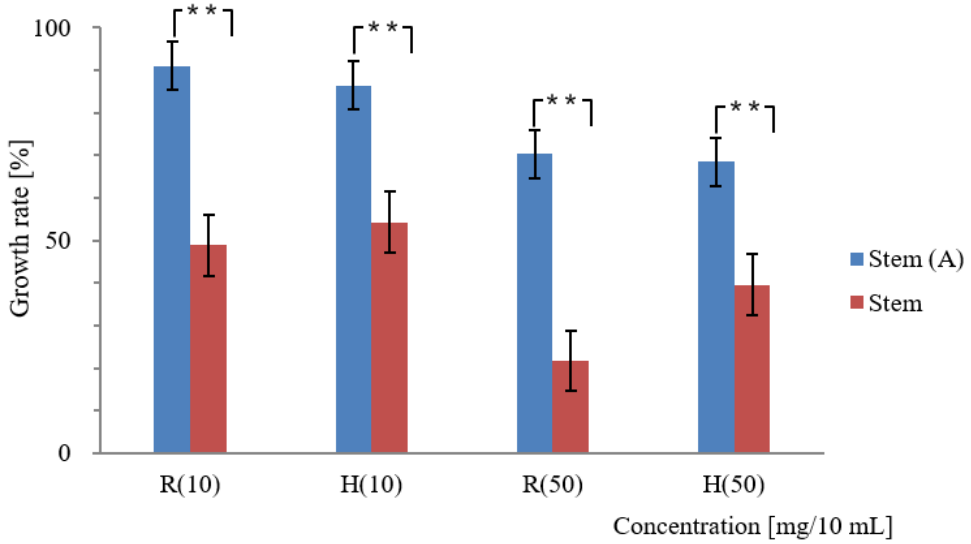


Figure 6. Sandwich method for the determination of the allelopathic effects of Platycodon stems. "A" indicates the results of the trial with activated carbon; results lacking identification represent the trial without the addition of activated carbon. At a concentration of 10 mg parts/10 mL agar, the difference between the results of the non-added and added activated carbon test areas was 0.5%, and this was a significant difference (<5.0%). Bars indicate standard deviation, ** indicates a significant difference at the 0.1% level by *t*-test.

3.3. Plant Box Method: Allelopathic Activity of Root Secretions from Platycodon

The results of the plant box method revealed a significant level of allelopathic inhibition in the roots of the test seedlings (Figure 7).

In the first test (after 60 days), repetition 1 exhibited an average inhibition rate of 28.6% in the radicle and 23.9% in the hypocotyls of the tested lettuce seedling. The inhibition rate was higher for the roots closer to the test seedlings and decreased with increased distance. The germination rate was 90.9%. Repetition 2 displayed average inhibition rates of 34.4% in the radicle and 10.4% in the hypocotyls; these also decreased with distance, and there was a germination rate of 100%. Repetition 3 showed average inhibition rates of 33.5% in the radicle and 14.9% in the hypocotyls, with a germination rate of 93.9%. Overall, the results from the three test repetitions indicated that the root secretions of Platycodon had a certain allelopathic inhibitory effect against the growth of the tested lettuce plant, with average inhibition rates of 33.1% in radicle and 16.4% in hypocotyls, and an average germination rate of 94.9%.

For the second test (after 90 days), repetition 1 exhibited average inhibition rates of 46.7% in radicle and 21.8% in hypocotyls, with a germination rate of 96.9%. Repetition 2 displayed average inhibition rates of 48.3% in radicle and 9.80% in hypocotyls, with a germination rate of 100%. Repetition 3 showed average inhibition rates of 41.7% in radicle and 12.4% in hypocotyls, with a germination rate of 100%. This indicated that the root secretions of Platycodon had average inhibitory effects of 45.6% on the radicle and 14.7% on the hypocotyls of lettuce, with an average germination rate of 98.9%.

In the third test (after 150 days), repetition 1 exhibited average inhibition rates of 57.7% in radicle and 22.3% in hypocotyls, with a germination rate of 90.9%. Repetition 2 displayed average inhibition rates of 55.9% in radicle and 28.6% in hypocotyls, with a germination rate of 87.9%. Repetition 3 showed average inhibition rates of 59.6% in radicle and 31.9% in hypocotyls, with a germination rate of 96.9%. Repetition 4 exhibited average inhibition rates of 57.7% in radicle and 20.4% in hypocotyls, with a germination rate of 93.9%. The results from these four test Repetitions indicated that the roots of Platycodon had an average inhibitory effect of 57.7% on the roots and 25.8% on the seedlings, with an average germination rate of 92.4% (Table 2).

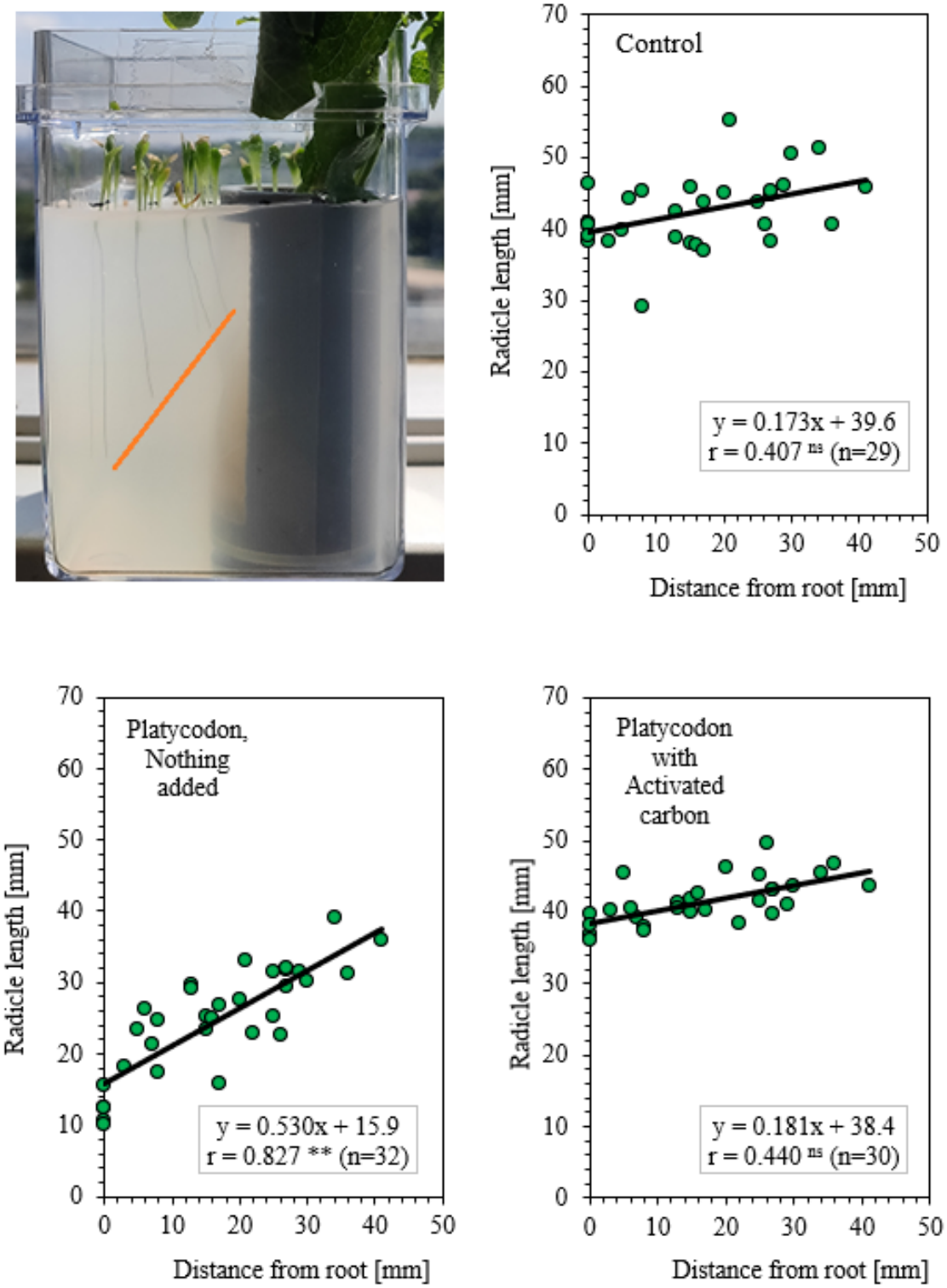


Figure 7. Evaluation of the allelopathic activity of root exudates by the plant box method. Activities were evaluated by first regression curve in the graph according to [38,39]. ** Indicates significant difference at the 0.1% level, ns means not significant by Pearson's correlation coefficient. Root elongation % was calculated by radicle length at distance zero from the root of donor.

Table 2. The absorption effect of activated carbon on allelopathic substances from various Platycodon components.

		10 mg			50 mg		
Date	**Part**	**R**	**H**	**G**	**R**	**H**	**G**
20 August	Leaf	90.2 ± 2.7	80.9 ± 1.8	100 ± 0.0	54.5 ± 3.1	62.6 ± 3.2	100 ± 0.0
	Root	64.3 ± 2.8	67.9 ± 1.8	100 ± 0.0	75.6 ± 2.5	72.8 ± 1.4	100 ± 0.0
	Stem	118.9 ± 3.6	106.2 ± 2.4	100 ± 0.0	68.5 ± 2.1	71.5 ± 1.6	93.3 ± 8.9
10 October	Leaf	83.2 ± 1.3	67.7 ± 0.9	100 ± 0.0	43.6 ± 1.6	45.9 ± 2.2	100 ± 0.0
	Root	80.1 ± 4.2	83.4 ± 3.4	100 ± 0.0	38.4 ± 1.7	50.9 ± 0.9	100 ± 0.0
	Stem	62.9 ± 1.8	66.6 ± 1.5	100 ± 0.0	71.9 ± 2.0	65.3 ± 1.8	100 ± 0.0
AIR	Leaf	13.3	25.7	0	50.9	45.8	0
	Root	27.8	24.3	0	43.0	38.1	0
	Stem	9.1	13.6	0	29.7	31.6	2.2

Values are the % of growth compared to the control; R: radicle; H: hypocotyl; AIR: average inhibition rate; G: germination ratio (%).

3.4. Effect of Activated Carbon on the Adsorption of Allelopathic Substances from Platycodon Roots

The results of using the plant box method to assess the absorption effect of activated carbon revealed that following the first test (after 90 days), repetition 1 exhibited an average inhibition rate of −34.2% in the radicle and −18.5% in the hypocotyls of the tested lettuce plant. This suggests that the addition of activated carbon significantly eliminated the inhibitory effect of root secretions from Platycodon. This effect promotes the growth of lettuce seedlings, with an average germination rate of 97.9%.

For the second test (after 150 days), repetition 1 displayed average inhibition rates of −10.4% in radicle and 5.1% in hypocotyls, while repetition 2 showed inhibition rates of 23.3% in radicle and 8.8% in hypocotyls. Repetition 3 exhibited inhibition rates of 3.0% in radicle and 2.0% in hypocotyls, and repetition 4 had inhibition rates of −13.7% in radicle and 3.8% in hypocotyls. The average inhibition rates for radical and hypocotyl growth throughout the four test repetitions were 0.8% and 9.1%, respectively, with a germination rate of 92.8% (Figure 8).

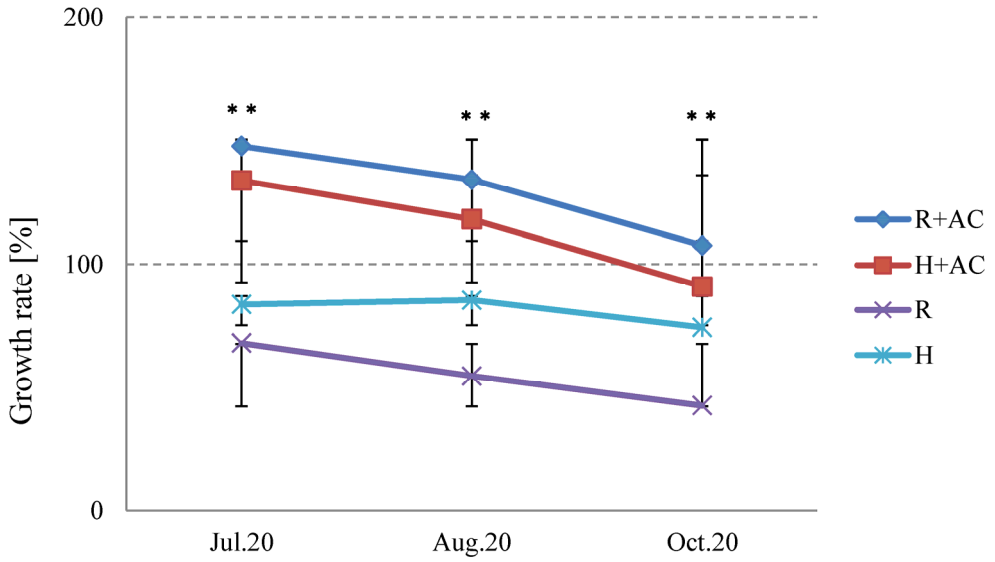


Figure 8. Effect of activated carbon on the allelopathic activity of root secretions from Platycodon. R: growth rate of young roots of test plants; H: growth rate of test plant seedlings; R+AC: growth rate of radicle of lettuce after adding activated carbon; H+AC: growth rate of hypocotyls after adding activated carbon. When the germination rate of the test plants had no added activated carbon (95.5%), the test area with activated carbon was added (94.8%). Bars indicate the SD. The significant difference

in the root growth rate between adding and not adding activated carbon was 0.3%, and the intentional difference in the seedling growth rate was 3.1%, which was less than 5.0%. Bars indicate standard deviation, ** indicates a significant difference at the 0.1% level by *t*-test. Dashed line means 100% growth to control, and over this line means promotion of growth.n

4. Discussion

4.1. Allelopathic Activity of Various Platycodon Components of Different Ages

In the context of issues with the continuous cropping of plants, the allelopathic substances released by the aboveground parts of plants are some of the main factors contributing to the formation of allelopathic autotoxicity. It has been noticed that with successive Platycodon cultivation, the yield and quality significantly decreased. The reason for this may be related to the autotoxicity of allelochemical release by this plant. The results of allelopathic tests using various parts of Platycodon showed a strong allelopathic inhibitory effect on the growth of lettuce radicles and hypocotyls. This inhibitory effect increased with increasing concentration. For instance, following the first test, the inhibition rate of the roots of lettuce seedlings was 73.9% at a concentration of 10 mg parts/10 mL agar, and it increased to 88.1% at a concentration of 50 mg parts/10 mL agar. This aligned with previous research findings [41].

As Platycodon has a growth cycle of 2–3 years, fallen leaves and allelopathic substances released by its aboveground parts accumulate in the topsoil layer over the years, potentially affecting the germination rate and growth of the next crop. The results of a previous survey also indicated a decline in the growth and germination rates of Platycodon with an increase in planting years, with the possibility of complete failure after six years of continuous cropping [42]. Therefore, the results suggest that allelopathic substances from various parts of Platycodon accumulate in the topsoil over several years, potentially leading to increasingly severe problems with continuous cropping.

Furthermore, the results from the three tests demonstrated that as the growth time was extended, the allelopathic effect of Platycodon was strengthened. For example, at a concentration of 10 mg parts/10 mL agar, the rate of inhibition of lettuce growth in the leaf repetition increased from 73.9% (first test) to 84.4% (third test). This indicated that as the growth time was extended, the concentration of allelopathic substances released by the plant increased, thus potentially resulting in an elevated concentration of these substances in the planting soil. In a previous study [43], water extracts from a whole Platycodon plant significantly inhibited seed germination, with an increase in the inhibition rate as the extract concentration rose. Therefore, allelopathic substances released by various parts of Platycodon may play a role in the formation of problems with continuous cropping. As the number of years of growth of Platycodon increases and problems with continuous cropping intensify, it can be inferred that allelopathic autotoxicity caused by the plant's self-released allelopathic substances may be one of the factors contributing to problems with continuous cropping.

4.2. Adsorption Effect of Activated Carbon and Its Impact on the Allelopathic Activity of Various Platycodon Components

In trying to solve problems related to continuous cropping in agriculture, crop rotation is a common method. However, recent studies have focused on mitigating the autotoxicity of the plant itself through methods such as improving soil nutrient composition and adsorbing allelopathic substances. Based on previous research results, this study tested the alleviating effect of activated carbon on continuous cropping problems associated with Platycodon.

The results of the sandwich method with activated carbon added to the culture medium showed that compared to the control group without activated carbon, the addition of activated carbon significantly alleviated the inhibitory effects of various parts of Platycodon on the growth of lettuce seedling roots and hypocotyls. Without activated carbon, at a concentration of 10 mg parts/10 mL agar of Platycodon leaves, the average rates of growth inhibition measured in lettuce seedlings and hypocotyls were 79.4% and 61.8%,

those for roots were 53.4% and 49.3%, and those for stems were 51.2% and 45.7%. However, with the addition of activated carbon, the inhibitory effects were significantly reduced. In the presence of activated carbon, the inhibition rates for leaves at a concentration of 10 mg parts/10 mL agar were 13.3% and 25.7%, those for roots were 27.8% and 25.8%, and those for stems were 9.1% and 13.6%. Similarly, the inhibition rate at a concentration of 50 mg parts/10 mL agar was also significantly reduced. The attenuation of the inhibitory effects with the addition of activated carbon increased with the concentration. This result aligned with the alleviating effect of activated carbon on problems with continuous cropping in asparagus and other vegetables observed in other studies [33].

In other studies, activated carbon was proven to exhibit certain alleviating effects on the continuous cropping problems for various crops. For asparagus, activated carbon caused reductions in the inhibition rate in the range of 61.5% to 58.7% [36]. Furthermore, activated carbon demonstrated a trend of alleviating inhibitory effects on leguminous crops and other vegetables, thus promoting plant growth [37]. Therefore, the results of this study are consistent with those of previous research, indicating that activated carbon has a certain alleviating effect on problems with the continuous cropping of Platycodon.

4.3. Allelopathic Activity of Root Exudation of Platycodon and its Role as an Obstacle to Continuous Cropping

In the process of plant growth, root secretions play a crucial role in the formation of problems with continuous cropping, as they involve interactions with soil microorganisms and soil nutrient components. Therefore, root secretions are essential in processes in which plants trigger allelopathic effects, which lead to problems with continuous cropping. Determining the allelopathic effects of root secretions is a key aspect of studying the causes of such problems. The results of this study indicate that root secretions of Platycodon had a certain allelopathic inhibitory effect on the growth of lettuce seedlings and hypocotyls, and this inhibitory effect gradually increased with the extension of the growth period. For example, in the results of the three tests, the average rate of root secretions' inhibition of the growth of lettuce seedlings showed a gradually increasing trend: 33.1% (first test), 45.6% (second test), and 57.7% (third test). As Platycodon has an optimal harvest time of 2–3 years, the secretions released by the roots during the long growth process may have accumulated in the soil, thus affecting the growth and germination rates of the next generation of Platycodon seedlings. Additionally, the allelopathic inhibitory effect of root secretions increased with concentration, indicating that the concentration of allelopathic substances accumulated in the soil may affect the growth and development of the next generation of seedlings.

An analysis of Platycodon root secretions revealed the presence of various bioactive substances, such as Platycodon saponin [2]. The secretion of these bioactive substances into the soil may affect the growth and germination of other plants or even Platycodon itself. Therefore, in future research, identifying the components of root secretions and conducting assessments of allelopathic effects will be crucial in unraveling the causes of problems with the continuous cropping of Platycodon. The preliminary results of this study confirm that the root secretions of Platycodon have a certain allelopathic inhibitory effect.

4.4. Adsorption Effect of Activated Carbon on the Root Secretion Allelopathy of Platycodon

In an experiment evaluating the allelopathic inhibitory effects of root secretions, the results after adding activated carbon revealed a significant alleviation in the inhibition of the roots and height of lettuce seedlings. For instance, without adding activated carbon, the average rates of inhibiting the radicle and hypocotyl length of lettuce were 35.5% and 18.9%, respectively. However, with the addition of activated carbon, the average inhibition rates were −23.9% and −4.7%, indicating not only the successful elimination of the inhibitory effect but also promotion of the growth of the tested plants. Previous research showed that activated carbon had a strong adsorption effect [40], so the reduction in allelopathic inhibitory effects may be attributed to the adsorption of allelopathic substances by activated

carbon. In this test, the addition of activated carbon led to growth rates surpassing those of the control group, suggesting that activated carbon not only adsorbed allelopathic substances but also had a certain growth-promoting effect. Therefore, activated carbon has the potential to be a material for alleviating the problems with continuous cropping associated with Platycodon. This finding provides robust support for future research to further explore the mechanisms by which activated carbon alleviates problems with continuous cropping in plants and its prospects for practical applications in agricultural production.

The results of the experiments in the laboratory revealed that the addition of activated carbon, diluted at a concentration of 1:25, significantly alleviated the allelopathy inhibition effect of Platycodon. In future studies, field experiments will be conducted to explore the optimal concentration in the field and investigate methods for application in Platycodon cultivation. As discussed earlier, activated carbon has been known to have mitigating effects on crop rotation disorders in vegetables such as asparagus and cucumber. There are also reports suggesting its effectiveness in alleviating crop rotation disorders in leguminous crops. Based on the results of these previous studies and the findings from our current experimental research, activated carbon seems to be a promising material for addressing the crop rotation issues associated with Platycodon.

5. Conclusions

This study analyzed the causes of problems with continuous cropping from the perspective of allelopathic effects and tested the mitigating effects of activated carbon reagents on problems with continuous cropping in Platycodon. This study provided scientific evidence for research related to alleviating continuous cropping problems in Platycodon. The use of materials such as activated carbon to mitigate autotoxicity in the cultivation process of Platycodon offers a new solution to alleviate continuous cropping problems, thereby enhancing productivity and facilitating the development of the Platycodon industry. Further laboratory and field studies are required to explore the optimal concentration of activated carbon reagents for mitigating autotoxic substances released by Platycodon plants. This research aimed to provide farmers with the best cultivation techniques and strategies.

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References

1. *Flora of China*. 1983, Volume 73, p. 77. Available online: https://www.iplant.cn/info/Platycodon%20grandiflorus?t=z (accessed on 9 October 2023).
2. Saeki, T.; Nikaido, T. Evaluations of saponin properties of HPLC analysis of *Platycodon grandiforum*. *J. Pharm. Sci.* **2003**, *123*, 431–441.
3. Wei, J.H.; Tu, P.F.; Li, G.; Wang, W.Q.; Wang, W.Q.; Yang, C.M.; Sui, C. Situation and Trends in Development of Chinese Medicinal Agriculture in China. *Mod. Chin. Med.* **2015**, *17*, 94–104.
4. Yong-Sook, K. Effects of *Platycodon grandiflorum* Feeding on Serum and liver Lipid Concentrations in Rats with Diet-induced Hyperlipidemia. *J. Nutr. Sci. Vitaminol.* **1995**, *41*, 489–491.

5. Wei, S.Z. Comprehensive development and utilization of platycodon resources. *J. Shaanxi Univ. Sci. Technol.* **2005**, *23*, 146–148.
6. Li, G.Q.; Bi, Y.W.; Chen, B.F.; Zhang, J.; Han, J.L.; Wang, Z.F. Research Advancesin Cultivationof Medicinal Plant *Platycodon grandiflorum*. *J. Agric.* **2016**, *6*, 55–59.
7. Zhang, Z.Y.; Lin, W.X. The allelopathic autotoxicity and consecutive cropping obstacles of medicinal plants. *J. Chin. Ecol. Agric.* **2009**, *17*, 189–196. [CrossRef]
8. Zhang, Z.L.; Wang, W.Q. The formation mechanism and prevention of continuous cropping obstacles of medicinal plants. *China Agric. Sci. Technol. Her.* **2009**, *11*, 19–23.
9. Sun, M.; Ye, L.Q.; Zhang, Z.L. Research progress on the causes and control of continuous cropping disorders of Panax Notoginseng. *J. Mt. Agric. Biol.* **2015**, *34*, 63–67.
10. Zhu, L.X.; Huo, X.H.; Sun, H.X.; Bi, S.; Ma, W.W. Effects of continuous *Platycodon grandiflorus* cultivation on soil physical-chemical and biological properties. *China J. Soil Water Conserv.* **2013**, *27*, 177–181.
11. Zhu, L.X.; Wang, J.H. Effects of controlled-release fertilizers on campanulaceae growth. *China J. Appl. Ecol.* **2010**, *21*, 2304.
12. Bao, L.; Bai, M.R.; A, D.Q.; Fujii, Y. Screening of allelopathic activity of plants growing in the desert area of Inner Mongolia Autonomous Region and discovery of Sea Buckthorn and Sweet annie as highly active plants and estimation of their active substances. *Jpn. J. Arid Land Stud.* **2019**, *29*, 1–10.
13. Molisch, H. *Der Einfluss Einer Pflanze auf die Andere-Allelopathie*; Fischer: Jena, Germany, 1937.
14. Tukey, H.B., Jr. Implicattions of allelopathy in agricultural plant science. *Bot. Rev.* **1969**, *35*, 1–16. [CrossRef]
15. Vives-peris, V.; De Ollas, C.; Gomez-cadenas, A.; Pérez-Clemente, R.M. Root exudates: From plant to rhizosphere and beyond. *Plant Cell Rep.* **2020**, *39*, 3–17. [CrossRef]
16. Wu, L.K.; Lin, X.M.; Lin, W.X. Advances and perspective in research on plant-soil-microbe interactions mediated by root exudates. *Chin. J. Plant Ecol.* **2014**, *38*, 298–310.
17. Wu, H.M.; Lin, W.X. A commentary and development perspective on consecutive monoculture problems of medicinal plants. *China J. Eco-Agric.* **2020**, *28*, 775–793.
18. Zang, A.H.; Gao, Y.; Xu, Y. Research progress on allelopathic effects of medicinal plants in China. *China J. Chin. Herb. Med.* **2011**, *42*, 1885–1890.
19. Wang, R.; Dong, L.L.; Xu, J.; Chen, J.W.; Li, X.W.; Chen, S.L. Progress in improving continuous monoculture cropping issues in *Panax ginseng* by controlling soil-borne diseases. *China J. Chin. Mater. Medica* **2016**, *41*, 3890–3896.
20. An, Y.; Yang, D.; Li, X.; Jin, X.J. Study on the effect and physiological mechanism of continuous cropping obstruction of *Pinellia ternata*. *Acta Agric. Borealioccidentalis Sin.* **2018**, *27*, 1017–1022.
21. Dai, L.; Singh, S.K.; Gong, H.; Tang, Y.; Peng, Z.; Zhang, J.; Wu, D.; Zhang, H.; He, D. Rhizospheric microbial consortium of *Lilium lancifolium* Thunb. causes lily root rot under continuous cropping system. *Front. Microbiol.* **2022**, *13*, 981615. [CrossRef]
22. Gu, L.; Niu, M.M.; Zheng, H.Y.; Wang, J.M.; Wu, L.K.; Li, Z.F.; Zhang, Z.Y. Effect of continuous cropping of *Rehmannia* on its morphological and physiological characteristics. *J. Chin. Med. Mater.* **2013**, *36*, 691–695.
23. Tan, Y.; Cui, Y.; Li, H.; Kuang, A.; Li, X.; Wei, Y.; Ji, X. Rhizospheric soil and root endogenous fungal diversity and composition in response to continuous *Panax notoginseng* cropping practices. *Microbiol. Res.* **2017**, *194*, 10–19. [CrossRef]
24. Liu, H.; Niu, M.; Zhu, S.; Zhang, F.; Liu, Q.; Liu, Y.; Liu, R.H.; Zhang, Y.P. Effect study of continuous monoculture on the quality of *Salvia miltiorrhiza* Bge roots. *BioMed Res. Int.* **2020**, *2020*, 4284385. [CrossRef]
25. Wu, H.; Wu, L.; Zhu, Q.; Wang, J.; Qin, X.; Xu, J.; Kong, L.; Chen, J.; Lin, S.; Umar, K.M.; et al. The role of organic acids on microbial deterioration in the *Radix pseudostellariae* rhizosphere under continuous monoculture regimes. *Sci. Rep.* **2017**, *7*, 3497. [CrossRef]
26. Zhang, J.; Fan, S.; Qin, J.; Dai, J.; Zhao, F.; Gao, L.; Lian, X.; Shang, W.; Xu, X.; Hu, X. Changes in the microbiome in the soil of an *American ginseng* continuous plantation. *Front. Plant Sci.* **2020**, *11*, 572199. [CrossRef]
27. Nishio, M. The current situation and causes of continuous cropping disorders. *Jpn. J. Chem. Biol.* **1985**, *23*, 582–589.
28. Zhang, Y.Y.; Zhu, D.W.; Wang, X.; Zhang, L.Z.; Shi, G.Y.; Zhou, J.; Liu, W. Properties of plant root exudates and obstacles for continuous cropping of medicinal plants. *China J. Hubei Agric. Sci.* **2014**, *53*, 1441–1444.
29. Qu, Y.T.; Zhang, Q.Q.; Yu, Y.F.; Duyxanale, S.; Cai, L.L.; Zhang, S.J.; Li, Y.F.; Li, Y.C. Research advances on mechanisms and preventions of the medicinal plants' continuous cropping obstacles from the perspective of rhizosphere microecology. *J. Zhejiang Univ. (Agric. Life Sci.)* **2022**, *48*, 403–414.
30. Huang, Y.F.; Zhang, E.H.; Zhang, X.H.; Wang, H.Z.; Wang, Q.; Liu, Q.L.; Cui, J.J.; Xu, S.R. Autotoxicity of water extracts from continuous cropping soil of *Lilium davidii* var. *unicolor* Salisb. *J. Northwest AF Univ. (Nat. Sci. Ed.)* **2020**, *48*, 84–93.
31. Liu, R.X.; Li, S.B.; Zhang, R.E.; Nie, C.P.; Li, Y.Y. Allelopathic effects of Platycodon aqueous extract on wheat seedlings. *China J. Biol.* **2014**, *31*, 28–32.
32. Gao, Z.R.; Zang, G.P.; Su, C.C.; Wang, Z.L.; Shi, Z.G.; Gong, Y.F.; Wei, Y.J. Research progress on the formation mechanism and mitigation measures of continuous cropping obstacles in medicinal plants. *China J. Anhui Agric. Sci.* **2023**, *51*, 21–25.
33. Nishihara, E.; Motoki, S. Agricultural use of activated carbon—New technology for soil purification, Nature and Science and Technology Series. *Jpn. Rural Cult. Assoc.* **2009**, 10–11.
34. Asao, T.; Hasegawa, Y.; Sueda, K.; Tomita, K.; Taniguchi, T. Autotoxicity of root exudates from taro. *Jpn. J. Sci. Hortic.* **2003**, *97*, 389–396. [CrossRef]

35. Asao, T.; Ohba, T.; Tomita, K.; Ota, K.; Hosoki, T. Effects of adding activated carbon to culture media with different dissolved oxygen concentrations for hydroponically grown cucumbers on plant growth and the number of harvested fruits. *Jpn. J. Hortic. Soc.* **1999**, *68*, 1194–1196. [CrossRef]
36. Motoki, S.; Nishihara, E.; Kitazawa, H.; Kyutoku, Y.; Uehara, T.; Yagasaki, K.; Sakai, H.; Shigemori, I. Effects of the application of activated carbon on soybean growth and yield in continuous legume fields. *Jpn. J. Crop Sci. Soc.* **2012**, *81*, 77–82. [CrossRef]
37. Nishihara, E.; Takahashi, S.; Hirata, T.; Nakano, T. Utilization of activated carbon for allelopathy avoidance technology in fava beans. *Kindergart. Sch. Misc.* **2006**, *75*, 569.
38. Fujii, Y. Establishment of an allelopathy assay method and the function of the active substance L-DOPA contained in *Mucuna*. *Jpn. Agric. Environ. Res. Inst. Rep.* **1994**, *10*, 115–218.
39. Fujii, Y.; Shibuya, T. Establishment of an activity evaluation method specific to allelopathy—Search for candidate plants by a mixed planting test in agar medium using a plant box. *Jpn. J. Weed Res.* **1992**, *37*, 156–157.
40. Motoki, S.; Nishihara, E.; Takahashi, N.; Limbers, H.; Shinohara, Y. Effects of astivated carbon to reduce allelopathy during raising the seedling stage. *Jpn. J. Hortic. Res.* **2007**, *6*, 603–609.
41. Zhai, Z.G. Yellow pines become green, chestnut production increases, etc. Research progress on plant root exudates. *Jiangxi J. Agric.* **2012**, *24*, 28–32.
42. Zhu, L.X.; Bi, S.; Liu, Y. Preliminary study on the autotoxicity of *Platycodon grandiflorum*. *China J. Lishizhen Med. Med. Res.* **2013**, *24*, 1992–1994.
43. Wang, Y.P.; Wang, H.T. Allelopathic substances secreted by plant roots and their environment in soil Behavior. *J. Soil Bull.* **2010**, *41*, 502–507.

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