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Investigation of *Solanum carolinense* Dominance and Phytotoxic Effect in *Festuca arundinacea* with Special Reference to Allelochemical Identification, Analysis of Phytohormones and Antioxidant Mechanisms

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Abstract: Exposure to invasive weeds in pasturelands may result in significant losses and toxicity in forage crops. These species may also contain a compound that may be toxic as well as beneficial depending upon the effect induced. The Ministry of Environment of the Republic of Korea has now recognized *Solanum carolinense* (Horsenettle)—an invasive weed species—as a potential threat to forage crops in pasturelands and to the entire agro-ecosystem. As a forage crop, *Festuca arundinacea* (Tall fescue) is one of the major economical crops and diets of livestock; in this study, the competition patterns of *Solanum carolinense* and *Festuca arundinacea* were examined with respect to their seeding ratios and growth periods. In addition, an extract from the root of *Solanum carolinense* (SCE) was prepared and treated at 2500 ppm and 5000 ppm in a *Festuca arundinacea* plant to observe its effect. The experimental results showed that as the growth period of the Horsenettle and the SCE treatment increased, the germination rate, plant height, root length, fresh weight, and dry weight of the tall fescue were significantly decreased. Moreover, the SCE treatment significantly increased the quantities of reactive oxygen species (O_2^- and H_2O_2), antioxidants (Catalase and Peroxidase), and endogenous phytohormones (Abscisic acid and Salicylic acid), and simultaneously decreased the superoxide dismutase content in the tall fescue shoots. Furthermore, we identified several glycoalkaloids from the SCE extract, among which Solanidan-3-ol, (3β,5α)' possessed a higher number (52%). Based on these results, we predicted that the Solanidan-3-ol, (3β,5α)' present in horsenettle has a major role in imposing phytotoxicity on agricultural crops. The glycoalkaloids in the *Solanum* species have been reported to possess both phytotoxic and therapeutic uses. Based on this concept, we believe that the compound available in *Solanum carolinense* could be used in developing crop protection or medicinal products through broader research. Conversely, our findings also showed the probable risk of horsenettle to the agro-ecosystem, especially in terms of forage production.

Keywords: bioherbicide; agro-ecosystem; allelochemical; crop protection; forage; sustainable agriculture; stress

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1. Introduction

Forage crops are one of the major diets of livestock and occupy vast areas used for commercial land cultivation [1]. They are considered the backbone of sustainable agriculture and the world economy [2]. Tall fescue is one of the most important forage plants due to its tolerance to drought, salinity, heavy metals, and excessive grazing, as well as its ability to minimize soil erosion. It is also widely cultivated as a forage base for beef, dairy, and wool production; thus, it can be a significant choice for large-scale cultivation [2,3]. This grass is often cultivated for slope stabilization in the Republic of

Korea [4]. However, the potential threat of invasion in forage croplands posed by invasive species may result in detrimental effects on forage production [5]. *Solanum carolinense* has been recognized as a significant threat to agricultural land and the ecosystem according to the report by the Ministry of the Environment of the Republic of Korea in 2021 (Appendix A). *Solanum carolinense* has a high capacity for spatial dispersal by natural means and different human-mediated pathways. As a result, the plant is likely to spread its range and infest cultivated land [6]. *Solanum carolinense* is expected to take over a wide range of locations in a short amount of time whenever it emerges due to its unpleasant qualities, e.g., herbivores find it unappealing to eat.

The glycoalkaloids present in *Solanum* sp. may result in both beneficial and detrimental effects, such as *Solanum chacoense* and *Solanum maglia* extracts, whose application has a strong inhibitory effect on mustard growth; however, the bioactive glycoalkaloids solasodine, solasonine, and solamargine isolated from *Solanum melongena* have medicinal properties [7,8]. A wide range of glycoalkaloids has been detected in *Solanum* plants [9]. *Solanum* species have also been reported to possess a strong allelopathic potential through varieties of allelochemical production [10]. In addition, *Solanum carolinense* has been reported to induce autoallelopathy in a few reports [11]. Exotic plants are characterized by having rapid fecundity and containing several allelochemicals that may also induce positive or negative allelopathy in the host plants [12,13]. The bioavailability and phytotoxicity of allelochemicals are determined by the outcome of the species interactions [14,15]. Moreover, allelochemicals are a novel class of herbicides that may be particularly useful in crop management [16]. Plants and other organisms interact through the release of allelochemicals and signaling chemicals into the environment [17,18]. As a result, allelochemicals help plants defend themselves against microbial attacks, herbivore predation, and/or competition with other plants, which prevents competing plants from adaptation [19,20]. The effect of allelochemicals in other species—especially on agricultural land—is important for optimum management and productivity [21,22]. Since *Solanum carolinense* has been previously reported on account of its detrimental effect as an invading species, identifying its allelochemicals and its effect on forage crops such as tall fescue are significant for sustainable agricultural practices.

Allelochemicals may induce secondary oxidative stress, thereby encouraging excessive reactive oxygen species in plants [23]. Conversely, some allelochemicals, such as those in garlic could improve the growth of plants by inducing a defense response system through regulating antioxidants and phytohormones [24]. Hence, understanding the underlying physiology, such as the reactive oxygen species, signaling through endogenous phytohormones, and the antioxidant system, helps to understand the defense mechanisms of plants [25,26]. Furthermore, the measurement of the invading tendencies of invasive species is crucial to assessing their impacts on pasture plants. The study of exotic weeds has led to the discovery of allelochemicals that can be used for a variety of agricultural applications [27,28]. However, little information is available about *Solanum carolinense*'s effects on agriculture. In this experiment, we will elucidate how we identified the allelochemical derivatives from *Solanum carolinense* and elaborate on its phytotoxic effect in *Festuca arundinacea*.

2. Materials and Methods

2.1. Plant Materials

The *Solanum carolinense* plants used in this experiment were collected from Daegu (35°53′43.5″ N 128°36′47.7″ E) in January 2020. The seeds of *Festuca arundinacea* were purchased from Da-nong Inc (Kentucky 31 tall fescue, Da-nong Inc., Namyangju, Korea). The *Solanum carolinense* used for extraction was freeze-dried using a freeze dryer (PVTFD20R, Ilshin Inc., Seoul, Korea).

2.2. Investigation of Competition Patterns between Festuca arundinacea and Solanum carolinense

2.2.1. Investigation of Competition Patterns according to the Seeding Ratio of *Festuca arundinacea* and *Solanum carolinense*

The total number of *Solanum carolinens:Festuca arundinaceae* seeds were sown, at a ratio of (So:Fe; 20:10, 10:10, and 10:20), into a pot (140 mm × 90 mm × 50 mm). The experiment was conducted in a greenhouse with temperature of 27 ± 4 °C and relative humidity 60~70%. Four weeks after sowing, a growth survey was conducted on plant height, root length, and fresh weight, and the sample was stored in an oven at 70 °C for 48 h to measure dry weight.

2.2.2. Investigation of Growth Patterns of *Festuca arundinacea* according to the Growth Period of *Solanum carolinense*

Solanum carolinense was transplanted into a pot 20, 40, and 60 days after sowing. One week after transplantation, 20 *Festuca arundinacea* seeds were sown into the pot containing 20-, 40-, and 60-day-old *Solanum carolinense* plants. After four weeks, the plants were harvested and plant height, root length, and fresh weight were measured, and the sample was stored in an oven at 70 °C for 48 h to measure dry weight.

2.3. Investigation of Phytotoxicity of SCE on Festuca arundinacea

2.3.1. Preparation of Crude Extract

A 350 g sample of lyophilized *Solanum carolinense* was suspended in 1150 mL of MeOH and kept in a shaker for 72 h. Afterward, it was filtered using filter paper (Advantec no. 2, Toyo Roshi Kaisha Ltd., Tokyo, Japan), and then concentrated in a round flask using a rotary vacuum evaporator (Eyela Rotary Vacuum Evaporator NN series, Eyela, Tokyo, Japan). The concentrated round flask was suspended using dH_2O and then freeze-dried using a freeze-dryer.

2.3.2. Treatment of Crude Extract in *Festuca arundinacea*

Fifteen grains of *Festuca arundinacea* seeds were sown in a pot (100 mm × 90 mm) and allowed to grow for four-weeks. A 10,000-ppm stock solution was prepared using crude extract and dH_2O. Accordingly, 2500 ppm and 5000 ppm of treatment solvent were prepared. A 5 mL pot^{-1} of each concentration was applied once a week for three weeks. After one week following harvesting of the last treatment sample, morphological parameters were measured and stored for biochemical analysis. Chlorophyll content was measured using portable CCM-300 Chlorophyll Contents Meter (ADC Bioscientific Ltd., Herts, UK).

2.4. Determination of Reactive Oxygen Species (ROS)

Reactive oxygen species are the result of stress suspected by the plant. These oxygen radicals alter the overall metabolism of the crops. Herein, to understand these complexities, we determine superoxide anion radical and Hydrogen peroxide measurements.

- Superoxide anion radical (O_2) measurement

The content of O_2^- was measured using the method of Navari-Izzo et al. [29]. In brief, the grounded sample was extracted using 10 mM potassium phosphate buffer (pH 7.8), 0.05% nitro blue tetrazolium (NBT), and 10 mM NaN_3. The mixture was suspended at 85 °C for 15 min using a water bath, immediately cooled, filtered, and measured at 580 nm using a spectrophotometer (Multiskan GO UV/Vis Microplate Spectrophotometer, Thermo-Fisher Scientific, Waltham, MA, USA).

- Hydrogen peroxide (H_2O_2) measurement

The content of H_2O_2 was measured using the protocol described by Jana and Choudhuri [30]. In brief, the sampled plant was harvested and immediately dipped in liquid N_2, grounded, and extracted with phosphate buffer (pH 7.5), and was then centrifuged at 8160× *g* for 20 min at 4 °C. The extract was suspended with 20% H_2SO_4 and dissolved in

0.5% of $TiCl_4$. The supernatant was collected through centrifugation at 8160× *g* for 20 min and measured at 410 nm using a spectrophotometer. The H_2O_2 content was calculated with a molar extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}.

- Visualization of ROS (H_2O_2)

H_2O_2 visual evaluation was performed according to the method described by Thordal-Christensenet et al. [31], namely, a DAB (3,3-diaminobenzidine) staining method using the shoot of the *Festuca arundinacea*. In brief, the sample was kept in the dark at room temperature while immersed in DAB solution (1 mg/mL; pH 3.8) for 12 h, and then the stained sample was heated using ethanol in a water bath at 90 °C for 10 min. After that, the sample was stored in 60% glycol for 1 to 2 min and evaluated visually.

2.5. Determination of Antioxidant Enzyme

- Superoxide dismutase (SOD) measurement

The SOD was measured by the methods described by Marklund and Marklund [32]. MeOH was added to the freeze-dried sample and extracted to prepare a sample solution. Tris-HCl buffer solution (50 mM Tris, pH 8.5) and 7.2 mM pyrogallol were added to dH_2O (AC) or each sample solution (AS) to react at room temperature for 10 min. Thereafter, 1 N HCl was added to measure absorbance at 420 nm using a spectrophotometer. The SOD-like activity was calculated by the following equation.

$$\text{SOD-like activity (\%)} = [1 - (AC/AS)] \times 100$$

- Catalase (CAT) measurement

CAT activity was measured by the method described by Aebi [33]. Frozen samples were extracted with a solution (50 mM Tris HCl, pH 7.0 3 mM $MgCl_2$, 1 mM EDTA, and 1% PVP), and supernatant was separated and mixed with the solution (0.1 M Phosphate buffer pH 7.0, 0.2 M H_2O_2). The degree of decomposition of H_2O_2 was measured at 240 nm using a spectrophotometer.

- Peroxidase (POD) measurement

The method described by Pütter [34] was used to confirm the activity of POD. The fresh sample was extracted using 0.1 M phosphate buffer (pH 6.8) and centrifuged at 19,386× *g* at 4 °C for 15 min. The supernatant was mixed with 0.1 M phosphate buffer, 50 μM pyrogallol, and 50 μM H_2O_2 and kept at room temperature for 5 min. Then, 5% of H_2SO_4 was mixed and measured at 420 nm using a spectrophotometer.

2.6. Quantification of Endogenous Phytohormones

- Abscisic acid (ABA) measurement

The extraction and quantification of ABA in plants were performed according to the method described by Kang, et al. [35]. In brief, a lyophilized sample was extracted using an extraction solvent of 95% isopropanol and 5% acetic acid and stirred at 1.15× *g* for 30 min. The extract was filtered and standard [(±-3,5,5,7,7,7-d6]-ABA 100 ng was added. The extract was concentrated using rotary vacuum evaporator, extracted with 1N Sodium hydroxide (NaOH), and then adjusted to pH 12 to 13 using a pH meter. Chlorophyll was removed using Dichloromethane (CH_2Cl_2) and the pH of the supernatant was adjusted to 2.5 to 3.5, followed by the addition of Ethyl acetate (EtOAc), and then the supernatant was collected. The extract was concentrated, extracted by phosphate buffer (pH 8.0) in polyvinylpolypyrrolidone (PVPP), and stirred for 1 h. The solution was filtered, and pH was adjusted to 2.5 to 3.5. EtOAc was added to collect the supernatant, concentrated in a vial with nitrogen gas, and methylation was performed with diazomethane and anhydrous CH_2Cl_2. The obtained solution was injected into GC-MS (6890N Network GC System and 5973 Network Mass Selective Detector: Agilent Technologies, Santa Clara, CA, USA) equipped with a 30 m × 0.25 mm (i.d.) HP-1 capillary column, and quantitative calculations

were performed by comparison to the standard curve derived from ABA-internal standard mentioned above.

- Salicylic acid (SA) measurement

Extraction and quantification of endogenous SA were performed by the method described by Seskar et al. [36]. A freeze-dried sample was extracted for 20 min by adding 90% MeOH. The extract was centrifuged at 9660× *g* and 4 °C for 15 min, and then the supernatant was collected. Then, 100% MeOH was added to the remaining pellet portion and extracted again using a centrifuge. After collecting the supernatant, it was concentrated using speedvac (SPD2030, Thermo Fisher Scientific, Waltham, MA, USA), 5% trichloroacetic acid was added, separation was performed in a centrifuge at 9660× *g* and 4 °C for 10 min, and the supernatant was collected. An extraction solution mixed at a ratio of 49.5%, cyclopentane and 1% iso-propanol was added to the supernatant; then, the supernatant was recovered and dried with nitrogen gas. The dried sample was dissolved with 1 mL of MeOH and 20 µL was injected into HPLC to perform quantitative analysis. HPLC conditions were used for the analysis and quantification of SA.

2.7. Identification of Allelochemical in Solanum carolinense

2.7.1. Herbicidal Activity Assay

A 10,000-ppm *Solanum carolinense* extract was prepared using dH_2O. Fifteen grains of *Festuca arundinacea* seeds were sowed on petri-dish (60 mm × 15 mm) layered with filter paper. After that, 2 mL of extract solution was inoculated and kept in an incubator at 30 °C for 7 days, and the germination rate and growth were investigated.

2.7.2. Isolation, Purification, and Identification of Allelochemicals

The *Solanum carolinense* root sample was ground and extracted with absolute methanol, concentrated using rotary vacuum evaporator, freeze-dried, and extracted using dH_2O. Solvent fractionation was performed with 100 mL of Hexane, Chloroform ($CHCl_3$), EtOAc, n-Butanol (BuOH), and dH_2O per 10 g of crude extract according to the polarity of the solvent. Each fractional layer was compared by performing a herbicidal activity assay by the method described above. Allelochemical separation and purification were performed on the $CHCl_3$ fraction, which had the highest growth inhibition rate among the solvent fractions. TLC (thin layer chromatography) was performed to set the appropriate mobile phase solvent for column chromatography. Column chromatography was carried out using silica gel (60, 0.040–0.063 nm, Merck Millipore, Darmstadt, Germany), and separation was carried out using a solvent in which MeOH, $CHCl_3$, and dH_2O were mixed at a ratio of 7:14:1. Consequently, three fractions—A, B, and C—were obtained. Each fraction was concentrated, and a germination inhibition assay was performed by the method described above; among them, column chromatography was performed on the B fraction, which had the highest growth inhibition rate with a solvent in which MeOH, $CHCl_3$, and dH_2O were mixed at a ratio of 6:14:1. Six fractions of BA, BB, BC, BD, BE, and BF were obtained and a herbicidal activity assay was performed. The BD fraction showed the highest activity to inhibit growth and was finally analyzed using GC-MS (Figure 1).

2.8. Statistical Analysis

All experiments were performed in three repetitions. Statistical significance analysis was expressed as mean and standard deviation using the Statistics Analysis System (SAS Software, Version 9.4, SAS Institute Inc., CARY, NC, USA) program. The difference between each processing sequence was tested at $p < 0.05$ through Duncan's Multiple Range Test (DMRT). Chemsketch version 12 was used to construct the molecular structure of the compound.

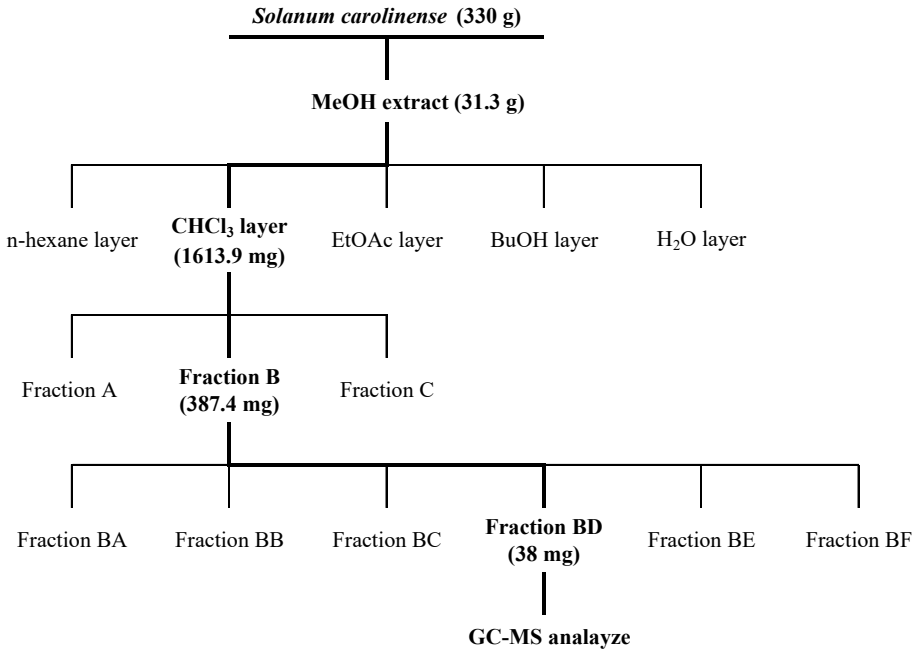

Figure 1. The process of separating and purifying allelochemical of *Solanum carolinense*.

3. Results

3.1. Investigation of Competition Patterns

3.1.1. Evaluation of Competition Patterns according to the Seeding Ratio

The *Festuca arundinacea:Solanum carolinense* growth rates were tested at ratios of 2:1, 1:1, and 1:2 (Figure 2, Table 1). The results showed that the germination rate, plant height, root length, fresh weight, and dry weight of *Festuca arundinacea* were significantly decreased when compared to the untreated group. This showed that *Solanum carolinense* has a significant dominance over the growth of *Festuca arundinacea*.

Figure 2. Effect of seeding ratio of *Solanum carolinense* on the growth of *Festuca arundinacea*.

Table 1. Effect of seeding ratio of *Solanum carolinense: Festuca arundinacea* on plant growth-promoting traits of *Festuca arundinacea*.

	Control	2:1	1:1	1:2
Germination rate (%)	81.7 ± 1.67 a	65 ± 0 b	56.7 ± 3.33 c	50 ± 2.89 c
Plant height (cm)	27.2 ± 0.92 a	18 ± 1.01 b	16.4 ± 0.79 b	15.6 ± 0.91 b
Root length (cm)	15.3 ± 0.88 a	8.6 ± 0.84 b	8.3 ± 0.58 b	10.2 ± 1.05 b
Fresh weight (mg)	1236 ± 174 a	110 ± 19.5 b	84 ± 13.3 b	57 ± 9.9 b
Dry weight (mg)	120 ± 17.51 a	14.8 ± 2.4 b	9.5 ± 1.79 b	9.1 ± 1.58 b

Each value represents the mean ± SD. Each data point represents the mean of at least six replicates. Different letters in the column after mean values represent the least significant differences at $p \leq 0.05$. The ratio is indicated as *Solanum Carolinense*: *Festuca arundinacea*.

3.1.2. Competition Patterns according to the Growth Period

The *Solanum carolinense* was grown for 20, 40, and 60 days and *Festuca arundinacea* was incorporated to observe the growth pattern. The germination rate, plant height, root length, fresh weight, and dry weight of *Festuca arundinacea* were significantly decreased in all treatment groups compared to the control. (Figure 3 and Table 2). According to this evidence, regardless of the growth phase, if *Solanum carolinense* dominates first, it has a significant inhibitory effect on *Festuca arundinacea*.

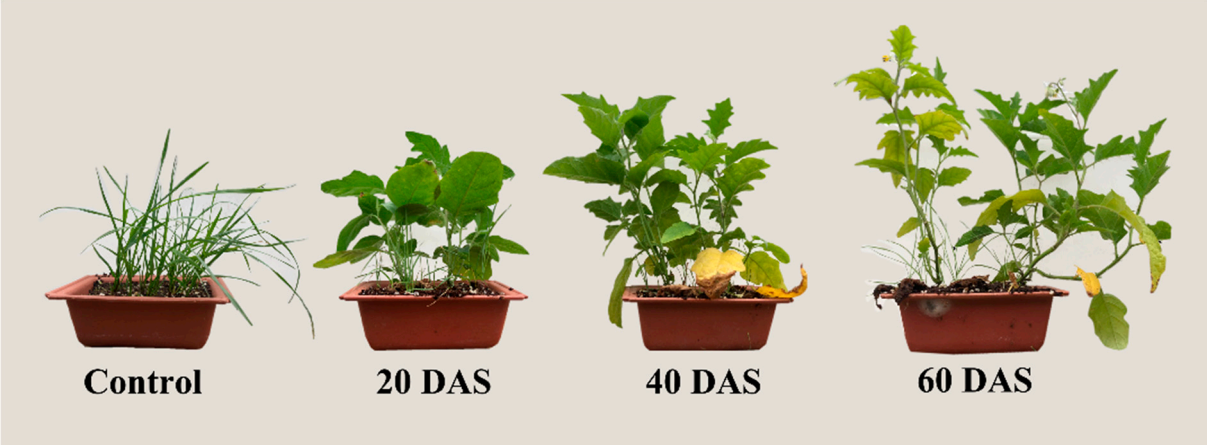

Figure 3. The effect of dominance of *Solanum carolinense* on the growth of *Festuca arundinace*.

Table 2. Effect of *Solanum carolinense* dominance on the growth of *Festuca arundinacea* at different time intervals.

	Control	20 DAS	40 DAS	60 DAS
Germination rate (%)	80 ± 0 a	61.7 ± 4.41 b	60 ± 2.89 b	60 ± 2.89 b
Plant height (cm)	22.9 ± 0.88 a	11.3 ± 0.48 b	9.8 ± 0.53 b	8.2 ± 0.39 b
Root length (cm)	12.8 ± 0.54 a	4.1 ± 0.34 b	3.9 ± 0.37 b	3.5 ± 0.44 b
Fresh weight (mg)	586.2 ± 58.7 a	55.1 ± 6.47 b	39.8 ± 13.74 b	17.9 ± 2.61 b
Dry weight (mg)	77.2 ± 11.4 a	5 ± 0.7 b	4.3 ± 1.59 b	2.8 ± 0.37 b

Each value represents the mean ± SD. Each data point represents the mean of at least six replicates. Different letters in the row after mean values represent the least significant differences at $p \leq 0.05$.

3.2. Investigation of Phytotoxicity of SCE on Festuca arundinacea

3.2.1. Effect on Morphological Characteristics and Chlorophyll Content

The results of the foliar treatment of SCE at 2500 and 5000 ppm concentrations showed a decrease in *Festuca arundinacea* height by 24.9% and 43.4%, fresh weight by 20% and 40%, and dry weight by 14.2% and 42.8%, respectively, when compared to the untreated plant (Figure 4, Table 3). The chlorophyll content decreased by 7.5% and 9.3% after treatment with the extract at 2500 ppm and 5000 ppm concentrations, respectively (Figure 5). Based on these results, it was predicted that SCE contains an allelochemical that inhibits the growth of *Festuca arundinacea*.

Table 3. Effect of SCE treatment on the growth of *Festuca arundinacea*.

Concentration (ppm)	Control	2500	5000
Plant height (cm)	32.8 ± 1.21 a	24.7 ± 1.54 b	18.6 ± 2.42 c
Fresh weight (g)	1.5 ± 0.06 a	1.2 ± 0.05 b	0.9 ± 0.04 c
Dry weight (g)	0.21 ± 0.005 a	0.18 ± 0.006 b	0.12 ± 0.005 c

Each value represents the mean ± SD. Each data point represents the mean of at least six replicates. Different letters in the row after mean values represent the least significant differences at $p \leq 0.05$.

Figure 4. Effect of SCE treatment on the growth of *Festuca arundinacea*.

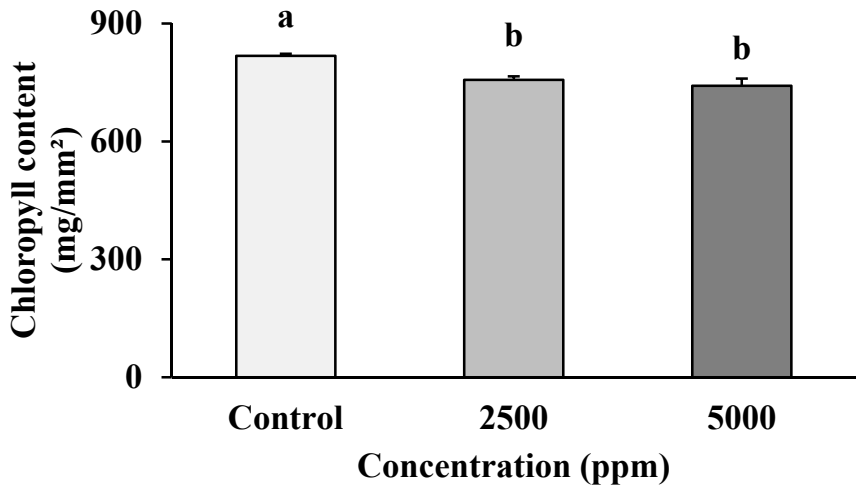

Figure 5. Effect of SCE treatment on chlorophyll content of *Festuca arundinacea*. Error bars represent standard deviations. Each data point represents the mean of at least six replications. Bars with different letters are significantly different from each other at $p \leq 0.05$.

3.2.2. Determination of Reactive Oxygen Species (ROS)

ROS act as signaling molecules regulating plant responses to biotic and abiotic stresses [37,38]. In our study, the crude extract treatments at 2500 and 5000 ppm significantly increased the Superoxide anion radical (O_2^-) content by 7.8% and 33.4%, respectively (Figure 6); similarly, the H_2O_2 content significantly increased to 33.3% and 85.8% (Figure 7). Moreover, the visual evaluation of H_2O_2 was observed after processing 5000 ppm of SCE in *Festuca arundinacea* using the DAB staining method. It was visually confirmed that the H_2O_2 content of the *Festuca arundinacea* leaves treated with the SCE was increased (Figure 8). The ROS radicals generate lipid peroxide, affect membrane permeability, and degrade DNA and proteins. Allelochemicals also depolarize the cell membrane, causing cellular disruption and cell death. Although all organisms require an appropriate level of ROS concentration, an excessive accumulation of ROS in plants can destroy chlorophyll and affect root development [39,40]. Based on these results, we can conclude that allelochemicals in the SCE induce detrimental effects in *Festuca arundinacea*.

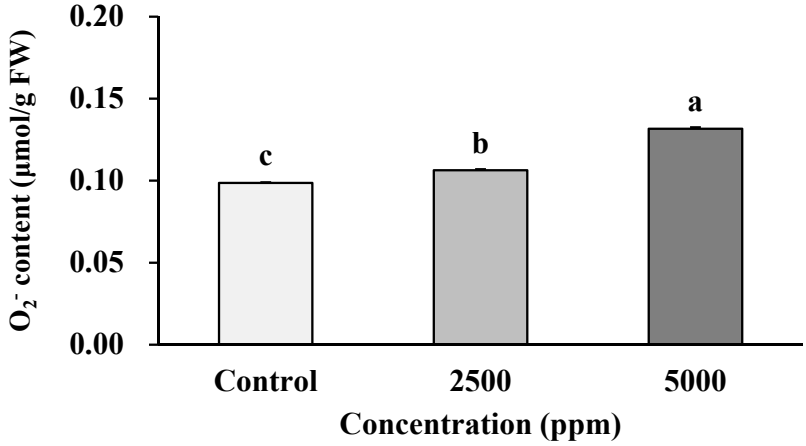

Figure 6. Effect of SCE treatment on the O_2^- content of *Festuca arundinacea*. Error bars represent standard deviations. Each data point represents the mean of at least eight replications. Bars with different letters are significantly different from each other at $p \leq 0.05$.

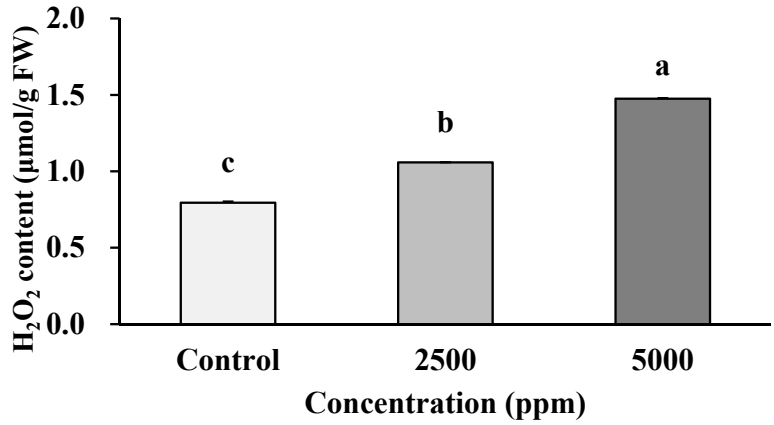

Figure 7. Effect of SCE treatment on H_2O_2 content of *Festuca arundinacea*. Error bars represent standard deviations. Each data point represents the mean of at least six replications. Bars with different letters are significantly different from each other at $p \leq 0.05$.

Figure 8. Visual evaluation of the adverse effect of SCE treatment on H_2O_2 content of *Festuca arundinacea*.

3.2.3. Determination of Antioxidant Enzyme

Antioxidants play a key role in conferring tolerance in plants under stress [41]. Here, as the SCE treatment increased in *Festuca arundinacea* from 2500 ppm to 5000 ppm, the SOD decreased by 38.3% and 80.5%, respectively; the CAT content increased by 34.1% and 64.5%, respectively; and the POD content increased by 6.5% and 12.7%, respectively (Figure 9A–C).

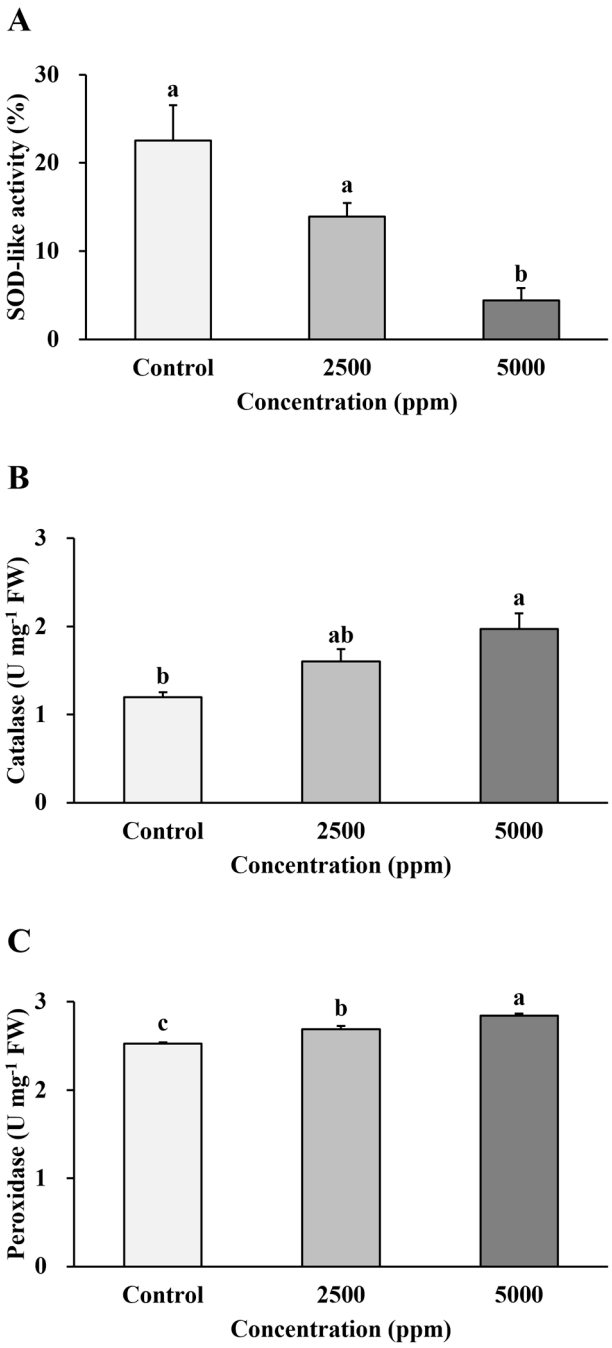

Figure 9. Effect of SCE treatment on the antioxidant content of *Festuca arundinacea* (**A**) SOD, (**B**) CAT, and (**C**) POD. Error bars represent standard deviations. Each data point represents the mean of at least six replications. Bars with different letters are significantly different from each other at $p \leq 0.05$.

3.2.4. Abscisic Acid (ABA) and Salicylic Acid (SA) Quantification

Allelochemicals affect plant growth and development by regulating phytohormones such as ABA and SA [42,43]. In this study, as the concentration of the extract increased to 2500 and 5000 ppm, the ABA content in *Festuca arundinacea* increased by 35.5 % and 49.7 % (Figure 10A); likewise, the SA content increased by 12.8% and 26.3%, respectively (Figure 10B), compared to the untreated group.

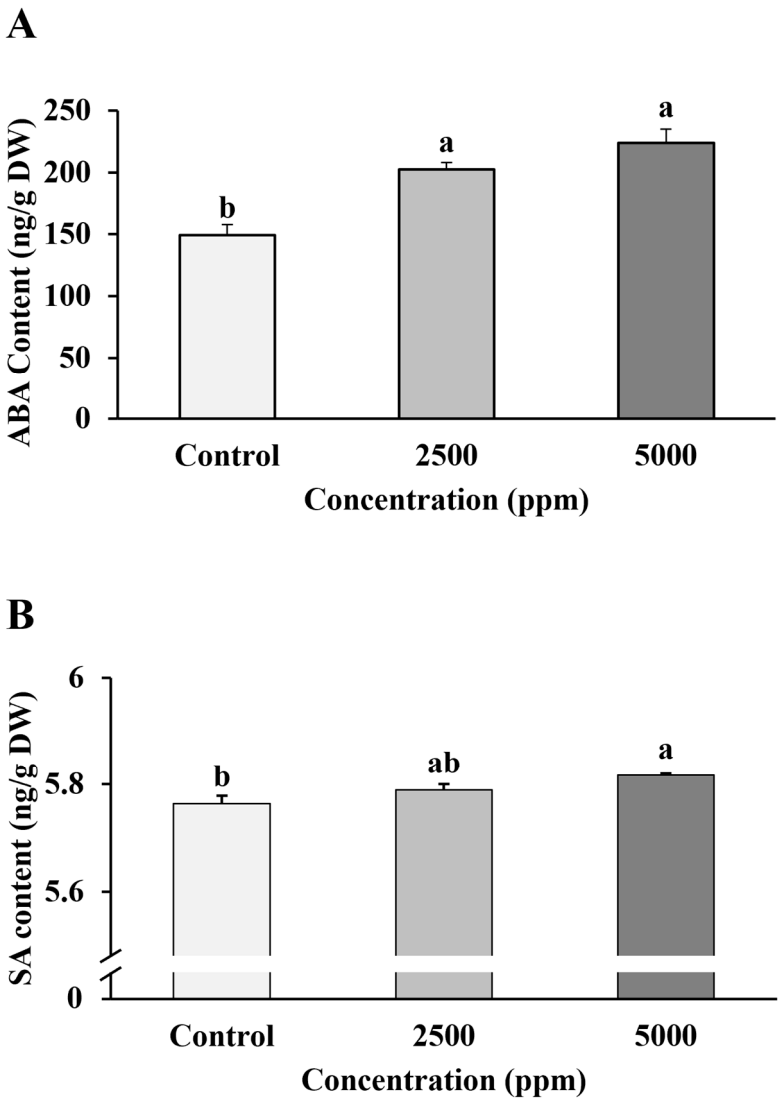

Figure 10. Effect of SCE treatment on abscisic acid (ABA) and salicylic acid (SA) content of *Festuca arundinacea*. Error bars represent standard deviations. Each data point represents the mean of at least three replications. Bars with different letters are significantly different from each other at $p \leq 0.05$.

3.3. Identification of Allelochemical in Solanum carolinense

As a result of performing the GC-MS analysis on the BD fraction, Solanidan-3-ol, (3β,5α) showed the highest concentration (51.96%). Several other glycoalkaloids such as 1H, 7H-[1,3]Benzodioxino[6,5-g][1,3]Benzodioxolo[5,6-a]quinolizine,9,10,15b,16-tetrahydro-5,15-dimethoxy-, (S) (11.5%), 5,5-Dimethylimidazolidin-2,4-dione (5.65%), solanidan-3-one (5.64%), and *D-Allose* (4.02%) were identified as major components (Figure 11 and Table 4)

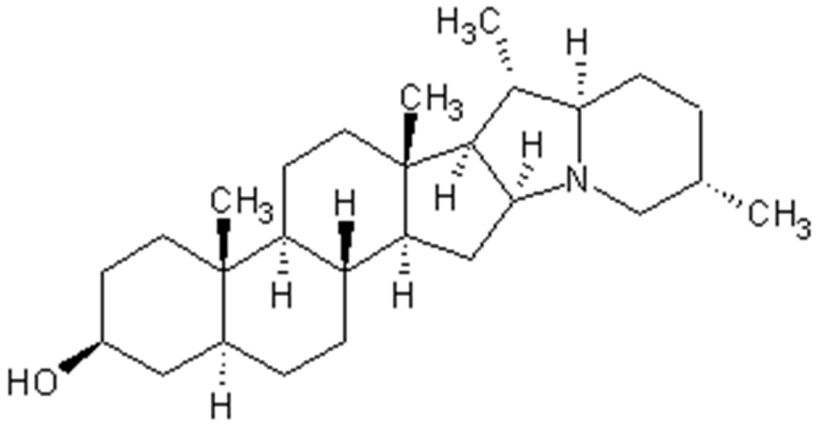

Figure 11. Molecular structure of Solanidan-3-ol, (3β,5α).

Table 4. Main compounds of SCEs analyzed by GC/MS.

No	Name of the Compound	Peak Area (%)
1	Solanidan-3-ol, (3β,5α)	51.96
2	1H,7H-[1,3]Benzodioxino[6,5-g] [1,3]benzodioxolo[5,6-a]quinolizine, 9,10,15b,16-tetrahydro-5,15-dimethoxy-, (S)	11.59
3	5,5-Dimethylimidazolidin-2,4-dione	5.65
4	solanidan-3-one	5.64
5	D-Allose	4.02

4. Discussion

Invasive plant species harm agriculture around the world, reducing crop productivity and quality [44]. However, the allelochemicals available in these species may be of significant use for defensive actions, such as herbicidal and insecticidal usages [19,45]. Since *Solanum carolinense* has been reported as a threat to agriculture and the eco-system, the current study demonstrates the growth pattern of *Solanum carolinense* and evaluates its toxic effect in the tall fescue type of forage grass. In addition, several allelochemicals were identified from *Solanum carolinense*, which might have induced a detrimental effect on tall fescue.

The current study demonstrated that the growth of tall fescue was significantly dominated by *Solanum carolinense* in all the seeding ratios as well as the increasing number of days after sowing. These enhanced the competition of the forage crops in response to *S. carolinense*. Our results are in line with Beeler et al. [46] who reported the adverse effect of *S. carolinense* on tall fescue's quality and yield. Moreover, the dominance of *S. carolinense* was reported in several other crops such as cereals especially wheat and maize, followed by soybean, potatoes and lucerne [6]. In general, one *Solanum carolinense* entity may produce 100 or more fruits, where each fruit may contain 40 to 170 seeds, and its roots are deeply lowered with a high starch content, making it difficult to achieve mechanical control [47]. In addition, chemical control is arduous due to its deeper root system [48]. Invading populations may remain undetected for years, and an invasion is only recognized once it has reached an unstable stage. Unfortunately, at this point, eradication is no longer an option, and slowing expansion rates are prohibitively expensive [49].

In addition to the high susceptibility of tall fescue to horsenettle invasions, our study also showed that when the tall fescue seedlings were treated with SCE, the treatment resulted in detrimental effects in plant growth and development. As a result, a further investigation was performed to identify the possible inhibitory compound in SCE. Following our investigation, we discovered numerous substances, the most prominent of which were the glycoalkaloids (Solanidan-3-ol, (3β,5α)) in SCE. In general, glycoalkaloids inhibit acetylcholinesterase (AChE) in living organisms and destroy biological membranes, and

are toxic to pathogens, insects, and humans [50,51]. This compound might have induced stress in the tall fescue plants, resulting in a significant drop in their growth and development. Similar results were shown by Fukuhara et al. [52], who reported that the steroidal glycoalkaloid arudonine inhibited the growth of *Lactuca Sativa*. Similarly, Sun et al. [9] reported that the glycoalkaloids solamargine/solasonine and chaconine/solanine retarded cucumber root growth. Moreover, our results are in line with Sołtys-Kalina et al. [53], who demonstrated the phytotoxicity induced by wild potatoes (*Solanum* sp.) against mustard.

Allelochemicals may be directly involved in the production of vulnerable ROS that stimulate oxidizing enzymes and increase free radicals [54,55]. In other cases, the allelochemicals might directly inhibit oxidizing enzymes in some way, leaving the plant vulnerable to oxidative damage [56]. Hence, the interplay of metabolomic aspects such as endogenous phytohormones (ABA and SA), antioxidants (CAT, POD, and SOD), and ROS (O_2 and H_2O_2) is crucial for understanding the resistance patterns of plants as they are directly involved in the cross signaling of plants [57–59]. The current study showed that the H_2O_2 and O_2 content was significantly elevated in tall fescue plants with the treatment of SCE. These results are supported by several authors; for instance, allelochemicals have induced ROS in various crops, from donor tobacco to acceptor lettuce [60,61], fescue to tomato [62], Chinese licorice to lettuce, and aromatic plants to wheat, wild oats, walnuts, and maize [26].

The plant activates an antioxidant defense system to assist in ROS detoxification under various abiotic stresses [63]. A variety of antioxidant systems counteract ROS accumulation in stress-induced cells, which include SOD, APX, GPX, GST, and CAT [64]. Our study showed that antioxidants such as CAT and POD were considerably elevated with the SCE treatment, while simultaneously dropping SOD levels. In general, O_2 is converted to H_2O_2 by superoxide dismutase; catalases then induce detoxification by converting $2H_2O_2$ to $O_2 + 2H_2O$ [65]. These phenomena were triggered by the influence of endogenous phytohormones such as ABA and SA [66].

The phytohormone ABA is involved in cross-signaling and SA in inducing systemic resistance in plants under stress [35,67]. The mechanism is involved in regulating antioxidants, modulating enzymes, coding amino acids, and triggering stress-responsive genes [68]. Our study showed that the SCE treatment elevated the ABA and SA levels of tall fescue. To a large extent, the higher ABA and SA levels signify the plant's ability to defend itself against the stress. Our results are in line with Han et al. [69], who showed that elevated ABA levels led to a higher accumulation of H_2O_2 in tall fescue under cadmium stress. However, the higher elevation of ABA and SA for defense against stress may cost significant losses in plant growth. Hence, the exogenous application of ABA and SA are generally preferred to ease the tall fescue defense mechanism, which has been reported by several authors on account of mitigating drought stress [70]; conferring heat tolerance [71]; improving phytoremediation [72]; strengthening the antioxidant system and photosynthesis [73]; enhancing shelf life, photochemical efficiency, and the transplant success rate [74]; and increasing gene coding and the production of enzymes [75].

5. Conclusions

Overall, our results demonstrated that *Solanum carolinense* has a high potential to cause agricultural crop invasions, resulting in adverse effects on the ecosystem. Moreover, the glycoalkaloid compounds in SCE induced phytotoxicity through increasing the number of ROS and activating defense-responsive mechanisms such as phytohormone signaling and the antioxidant system. This evidence may provide significant insights into developing strategies for the management of invasive species and forage crop production. Moreover, from the perspective of the beneficial and detrimental effects of *Solanum* sp. glycoalkaloids, the compound that we have discovered from *Solanum carolinense* extract may be valuable for future research to ease the scientific burden involving the identification of the plants.

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Appendix A

Information regarding different exotic species as reported in the database of the Ministry of Environment, South Korea, is available through the following link: (law.go.kr) (accessed on 5 July 2021).

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