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Growth inhibition of six test plants by *Stephania japonica* (Thunb.) Miers leaf extracts is an indication of allelopathic activity

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Abstract

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For sustainable agriculture, exploiting allelochemicals from different groups of plants to treat noxious weeds is increasing. In this study, we assessed the possible allelopathic activity of aqueous methanol extracts of *Stephania japonica* leaves at six concentrations on the seedling growth of six test plants. The results showed a significant variation in growth of the test plants when treated with the extracts ($p < 0.05$). At 0.3 g dry weight (DW) equivalent *S. japonica* leaf extract per mL, the seedling growth of the test plants were completely inhibited except for the barnyard grass shoots, and the inhibitory activity was plant species and concentration dependent. The seedlings of the dicots were the most sensitive to *S. japonica* extracts with the amount of extract required for 50% shoot and root growth suppression (I_{50} value) in the range of 0.003–0.008 g DW equivalent *S. japonica* extract per mL. In comparison, the I_{50} values for the monocots were 0.003–0.045 g DW equivalent *S. japonica* extract per mL. The I_{50} values show the shoot growth of the cress and lettuce and the root growth of the cress, alfalfa, and timothy were the most sensitive to the *S. japonica* leaf extracts, while barnyard grass was the least sensitive. These results showed that *S. japonica* probably has allelopathic activity that suppressed the growth of the test plants, indicating that it could be used as a biological tool to combat weeds. However, further research is required to identify and to isolate the inhibitory substances of *S. japonica*.

Keywords: Stephania japonica; allelopathic activity; biological tool; weed control

Introduction

Weeds are the most common and serious hindrance to crop production because they compete with crops for water, soil, nutrients, light, space, and other resources (McErlich & Boydston, 2013). Of the different agricultural pests, weeds cost the most to control throughout the world because they cause higher yield losses and increase production costs for farmers compared with other pests. It has been reported that weed infestations cause about 34% loss of major crop yields worldwide (Jabran et al., 2015). To manage this pest, farmers use synthetic herbicides because of their easy availability and low cost compared with the mechanical control of weeds, but chemical control leads to environmental pollution. Indiscriminate use of these herbicides results in weeds developing resistance to them (Mazumder, 2011; Daniel et al., 2013; Heap, 2014). It has been determined that 514 species of weed have developed a high level of resistance to many herbicides due to intensive use, and furthermore, they pose risks to wildlife and human health (Heap, 2019). There-

fore, the scientific community has been looking for alternative methods to manage weeds for ecological agriculture.

Allelopathy, a biological phenomenon in which plants possessing natural biochemicals (called allelochemicals) negatively or positively affect the growth and developmental of other plants, is now a predominant and effective strategy to control weeds for endurable agricultural production (Arroyo et al., 2018). Negative allelopathic research (Regiosa et al., 2006; Willis, 2007; Suzuki et al., 2019) in the twenty-first century has gained traction for sustainable agriculture; the research focuses on evaluating the allelopathic activity of various plants, isolating their natural metabolites, and determining the implication of their use against harmful plants, so that the plants possessing allelochemicals ultimately become natural tools for managing weeds.

Stephania japonica (Thunb.) Miers is a climbing plant in the family Menispermaceae. This annual plant is widely used in South Asian countries as a traditional treatment for many diseases, and it has been used without any basis on scientific evidence, although applications vary by region. The common name of this plant is tape vine, snake vine, or lotus-leaved vine (Korea National Arboretum, 2015). The distribution of this vine is widespread and is usually found in Bangladesh, Japan, Korea, China, Taiwan, Philippines, Thailand, Malaysia, Australia, India, and Sri Lanka. This species was first identified in Japan; hence the species name is "japonica" (Robinson, 2003). Its leaves are peltate (leaf attached to the stem by a long petiole) 2–12 cm long, 3–10 cm wide, and circular to ovate or triangular. Its pale green flowers are 4–8 cm long, and flowering occurs in July to September through summer. The fruit is red or orange drupe and oval, 2–5 mm long. A dioecious climber, it has slender stems without prickles.

In Bangladesh, *S. japonica* is used to treat many ailments including fever, sleep disturbance, cancer, asthma, edema, and bone fractures (Kirtikar & Basu, 1987; Jahan et al., 2010). In particular, the crushed leaves of *S. japonica* are used to treat body pain (Seraj et al., 2013), and warmed *S. japonica* leaves are used for rheumatism (Rahman *et al.*, 2007). In Indonesia, green jelly for human consumption is made from the leaves of this plant. Moreover, there have been many reports that this plant possesses biochemical constituents such as alkaloids, steroids, and fats (Senthamarai et al., 2012), and bioactivities such as anti-inflammatory, anti-nociceptive, antioxidant, anti-diarrheal, insecticidal, and antidiabetic (Ahmed et al., 2011; Sultana et al., 2012; Moniruzzaman et al., 2016; Ahmed, 2016; Islam & Khatun, 2017). Minhajur et al. (2012) also described *S. japonica* having an anti-microbial and cytotoxic effect in brine shrimps. However, there has been little research on the allelopathic activity of *S. japonica* and the allelochemicals responsible for its phytotoxicity. Therefore, this experiment was conducted to determine the allelopathic potential of *S. japonica*, which could be a candidate for eco-friendly management of weeds.

Materials and Methods

Source of plant samples

Stephania japonica (Thunb.) Miers leaves used in this experiment were obtained from the Sirajganj district of Bangladesh (24° 27′ 27.76″ N, 89° 42′ 28.87″ E) from July to August 2020. The samples were washed under running tap water to remove dirt and dried in a shady place. The dry leaves were ground into a powder using a mechanical grinder (Retsch, GM 200 Laboratory grinder, D-42781 Haan, Germany) and then kept in the refrigerator until analysis.

Test plant species

Three monocots [Italian ryegrass (*Lolium multiflorum* Lam.), barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv), and timothy (*Phleum pratense* L.)] and three dicots [alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), and lettuce (*Lactuca sativa* L.)] were used as test plants, and growth assays were conducted using these plants. The test plants were selected for their responsiveness to allelopathic extraction, growth behavior, and their wide distribution (Hossen & Kato-Noguchi, 2020).

Preparation of plant extracts

The leaf powder (100 g) of *S. japonica* was extracted for 48 h at 25 ± 2°C in 70% (v/v) aqueous methanol, to a final volume of 1000 mL. It was then filtered using filter paper No. 2 (125 mm; Toyo Roshi Kaisha Ltd., Japan). The residue was again inundated with 1000 mL methanol and filtered for 24 h using the same filter paper. The two filtrates were combined and evaporated to dryness using a rotavapor at 40°C (Rotary evaporator Model RE 200, Yamato Scientific Co. Ltd. Japan). Crude methanol extracts were then prepared for bioassay.

Bioassay

The crude extracts (1.5, 4.5, 15, 45, 150, and 450 µL) of the *S. japonica* leaves were diluted with 250 mL methanol to produce six concentrations of 0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g DW equivalent *S. japonica* extract per mL. An aliquot of each concentration of the extracts was added to each Petri dish (28 mm) containing filter paper No. 2 (Toyo Roshi Kaisha Ltd.). The Petri dishes were placed in a draft chamber to evaporate the methanol. Subsequently,

an aqueous solution of 0.6 mL of 0.05% polyoxyethylene sorbitan monolaurate (v/v) (Tween 20; Nacalai Tesque, Inc., Kyoto, Japan) surfactant was added to the Petri dishes for the uniform absorption of the extracts. Germinated seeds (n=10) of barnyard grass, Italian ryegrass, and timothy (seeds were germinated in the dark at 25°C and 72, 60, and 48 h, respectively) and viable seeds (n=10) of alfalfa, cress, and lettuce were arranged in each prepared Petri dish. Petri dishes with only Tween 20 were used as control. The Petri dishes were kept in an incubator for 48 h in darkness at 25°C. The length of the shoots and roots of the test plants were then recorded to calculate the growth inhibition of the test plants and the concentrations needed for suppressing 50% growth (I_{50} value) of each test plant species.

Statistical analysis

The experiment was conducted in a completely randomized design (CRD) with three replications repeated two times, and ten seeds were used for each replication (n=60). The recorded data was analyzed using SPSS version 16.0 (IBM Corp, 2007). Analysis of variance (ANOVA) and significant differences were determined using post-hoc Tukey's test at p=0.05. A regression equation was used to calculate the I_{50} value of each test plant. The bivariate Pearson Correlation was used to measure the correlation (correlation coefficient, r) between the *S. japonica* leaf extracts and the growth of the test plants.

Results

The inhibition of the shoot and root growth of the test plants treated with different concentrations of the aqueous methanol extracts of *S. japonica* is presented in Figure 1, Figure 2 and Figure 3.

The extracts significantly inhibited shoot growth at ≥0.1 g DW equivalent *S. japonica* extract per mL, and root growth was mostly inhibited at all concentrations for the six test plants (Figure 2 and Figure 3).

With exposure to 0.03 g DW equivalent *S. japonica* extract per mL, the shoot and root growth of alfalfa, cress, lettuce, Italian ryegrass, barnyard grass, and timothy were inhibited to 17.64, 3.14, 16.67, 51.90, 108.50, and 28.78 of control, respectively, and 23.10, 2.90, 11.01, 31.88, 32.06, and 13.14% of control, respectively. However, a significant difference was observed between control and the shoot and root growth of all the treated plants when the concentration was 0.1 g DW equivalent *S. japonica* extract per mL. The shoot growth of alfalfa, timothy, Italian ryegrass, and barnyard grass was inhibited by 95, 94, 89, and 30%, respectively, while the cress and lettuce shoots were completely

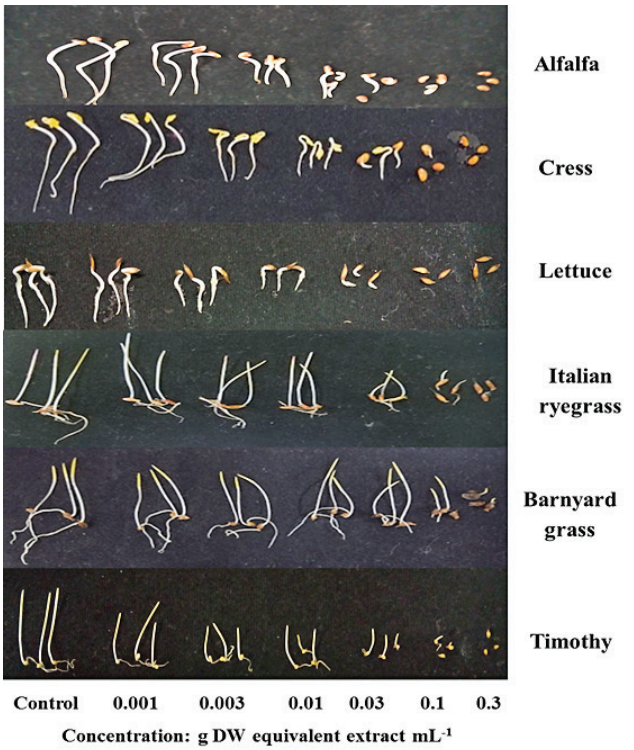

Fig. 1. Effect of treatment with different concentrations of *S. japonica* leaf extract (control, 0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g DW equivalent extract per mL) on the test plant species (alfalfa, cress, lettuce, Italian ryegrass, barnyard grass, and timothy)

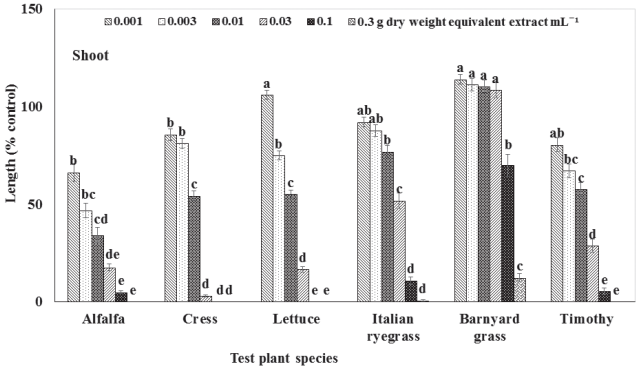

Fig. 2. Shoot growth inhibition of six test plants due to different concentrations of *S. japonica* leaf extract (0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g DW equivalent extract per mL)

Mean ± SE from two separate experiments are presented where each treatment was replicated 3 times (10 seedlings per treatment, n=60). Standard error of the mean is depicted by a vertical bar. The different letters in each panel illustrate a significant difference by Tukey's HSD test ($p < 0.05$)

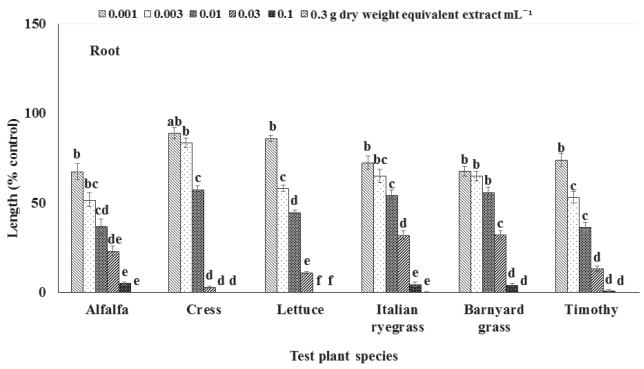

Fig. 3. Root growth inhibition of six test plants due to different concentrations of *S. japonica* leaf extract (0.001, 0.003, 0.01, 0.03, 0.1, and 0.3 g DW equivalent extract per mL)

Mean ± SE from two separate experiments are presented where each treatment was replicated 3 times (10 seedlings per treatment, n = 60). Standard error of the mean is depicted by a vertical bar. The different letters in each panel illustrate a significant difference by Tukey's HSD test ($p < 0.05$)

inhibited. At the same concentration, the root growth of alfalfa, Italian ryegrass, and barnyard grass was inhibited by 95%, while the cress, lettuce, and timothy roots were completely inhibited. The degree of inhibition increased as the concentration increased, and individual plants showed different responses. The extracts at 0.3 g DW equivalent *S. japonica* extract per mL completely inhibited the shoot growth of all the test plants excluding barnyard grass, which was inhibited by 87.64% compared with control, whereas no root growth was observed in any of the test plants treated with the same concentration. The correlation coefficient (r) values showed a significant negative association between the *S. japonica* extract concentration and the shoot and root growth of the test plants (Table 1).

Table 1. The correlation coefficient between the seedling growth of the six test plants and the concentrations of the *S. japonica* extracts

Test plant species	Correlation coefficient (r)	
	Shoot	Root
Alfalfa	−0.861**	−0.858**
Cress	−0.933**	−0.929**
Lettuce	−0.955**	−0.972**
Italian ryegrass	−0.900**	−0.937**
Barnyard grass	−0.667**	−0.937**
Timothy	−0.905**	−0.930**

** Correlation is significant at the 0.01 level

The r values ranged from −0.667 to −0.955 for the shoots and −0.858 to −0.972 ($p < 0.01$) for the roots. In addition, the required concentration for 50% inhibition of the shoot and root growth of the six plant species varied from 0.003 to 0.045 and 0.003 to 0.022 g DW equivalent *S. japonica* extract per mL, respectively (Table 2).

Table 2. Required concentrations of the *S. japonica* leaf extracts for 50% shoot and root growth inhibition (I_{50} values) of the six test plants

Test plant species	I_{50} (g dry weight equivalent extract mL^{-1})	
	Shoot	Root
Alfalfa	0.008	0.003
Cress	0.003	0.003
Lettuce	0.003	0.006
Italian ryegrass	0.010	0.012
Barnyard grass	0.045	0.022
Timothy	0.013	0.003

For the monocots, the concentration needed was 0.010–0.045 and 0.003–0.022 g DW equivalent *S. japonica* extract per mL for the shoots and roots, respectively, which was relatively higher than for the dicots at 0.003–0.008 and 0.003–0.006 g DW equivalent *S. japonica* extract per mL, respectively, indicating that the dicot plants were more sensitive to the *S. japonica* leaf extracts than the monocots. Based on the I_{50} values, cress was the most sensitive and barnyard grass was the least sensitive to the plant extracts. Thus, the response of the test plants to the *S. japonica* leaf extracts in the order of most sensitive to least sensitive was the cress seedlings followed by lettuce, alfalfa, timothy, Italian ryegrass, and barnyard grass.

Discussion

The results showed that the *S. japonica* leaf extracts significantly inhibited the growth of the test plant seedlings ($p \leq 0.05$) (Figure 2 and Figure 3). The present experiments were conducted with no additional nutrient solutions, and all the individual Petri dishes were incubated under dark conditions as described in the materials and methods section. Nutrients are not required during the initial growth stage because most nutrients are obtained from the seed reserves, and light is not inevitable in this growth stage (Fuerst & Putnam, 1983; Ashrafi et al., 2009). Thus, such growth inhibition of the test plant species by the *S. japonica* extracts may be due to an allelopathic reaction rather than nutrient media, competitive influences, or environmental variables. According to previous studies, *S. japonica* possesses medicinal and bi-

ological properties from bioactive compounds such as aknadinine, epistephanine, and hernandifoline (Senthamarai et al., 2012). Several studies have reported that many medicinal plant species contain biological compounds that possess allelopathic potential, which suppressed the growth of test plants (Rawat et al., 2016; Shinwari et al., 2017; Bari et al., 2019; Rob et al., 2021). Moreover, the results of the present study revealed that the growth inhibition of all the test plant species by the *S. japonica* leaf extracts was concentration related (Table 1). Our results are similar to the results of studies on the extracts of several medicinal plants such as *Caesalpinia mimosoides* Lamk (Boonmee et al., 2018), *Rhanterium epapposum* and *Salsola imbricata* (Al-Harbi, 2018), and *Acacia catechu* (L.f.) Wild (Hossen & Kato-Noguchi, 2020), which showed that inhibition of the seedling growth of test plants increased with increased extract concentration. Travlos et al. (2008) and Gomaa et al. (2014) also found that increasing the concentrations of extracts may lead to intensifying the action of inhibitory substances. Therefore, the concentration-dependent responses of the test plant species in this study indicated that the *S. japonica* extracts possess allelopathic potential from phytotoxic substances.

The I_{50} values of the six test plant species indicated the cress seedlings (I_{50} = 0.003 g DW equivalent *S. japonica* extract per mL) were the most sensitive to the *S. japonica* leaf extracts, whereas the barnyard grass seedlings (I_{50} = 0.022–0.045 g DW equivalent *S. japonica* extract per mL) were the least sensitive (Table 2). Such species-dependent responses to plant extracts have been recorded in several studies (Das & Kato-Noguchi, 2018; Krumsri et al., 2019; Zaman et al., 2021; Li et al., 2021). The differences in susceptibility of plant species to phytotoxic substances could result from differences in physiological and biochemical processes in plant species (Kobayashi, 2004; Sodaeizadeh et al., 2009). For example, dissimilarity in seed size, shape, and the permeability of seed coats might be one reason related to the absorption of phytotoxic substances, resulting in variable responses among test plant species (Zakaria & Razak, 1990; Hassan et al., 2012). In this study, the *S. japonica* leaf extracts markedly inhibited both the shoot and root growth of all the test plant species. Such growth inhibition of test plants may be caused by disrupting cell division, which is associated with the inhibition of DNA-RNA synthesis, energy production needed for mitosis division, as well as interrupting enzymatic activity (Kilhman, 1966; Sato et al., 1982; Inderjit & Keating, 1999; Cai & Mu, 2012; Rial et al., 2014).

These results suggest that the *S. japonica* leaf extracts contain phytotoxic substances that reduced the seedling growth of the six test plants. Therefore, the results of this study indicate the feasibility of using *S. japonica* leaf extracts as an environmentally friendly herbicide to manage weeds in agriculture. However, further research is needed to isolate and chemically profile the active inhibitory substances in *S. Japonica* leaf extracts.

Conclusion

Stephania japonica leaf extracts significantly inhibited the growth of the test plants, which could be linked to the action of plant inhibitory substances. The level of growth inhibition depended on the extract concentration and the test plant species. Our findings indicated that *S. japonica* possesses allelopathic substances that were responsible for the restricted growth of the test plants. Therefore, *S. japonica* could be used as part of a biocontrol strategy that could serve as an alternative to synthetic herbicides.

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