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Research Article

Allelopathic potential effects of *Rhazya stricta* plant extract on growth control of *Cuscuta campestris* weed seedlings

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ABSTRACT- Among weeds, parasitic plants have more profound effects on host plants. Due to its wide geographical distribution, high host range, and inefficient management methods, the Cuscuta (*Cuscuta campestris*) plant has become one of the most damaging parasites. The present study aimed to control the Cuscuta weed biologically. To this end, an experiment was conducted to investigate the allelopathic effects of the extract of the *Rhazya* Stricta plant on the control of this weed population in the laboratory of Velayat University of Iranshahr. The experiment was conducted as a completely randomized design with three replicates. In this experiment, negative allelopathic effects of different extracts of the *R. stricta* plant including acetone, aqueous, methanolic, ethanolic, and hydroalcoholic extracts on the Cuscuta were investigated in comparison with the distilled water (as control treatment). The results of this study revealed that the aqueous extract of *R. stricta* significantly decreased the germination percentage, germination vigor, germination rate, root length, and stem length of the Cuscuta compared to those of other treatments. Also, the other extracts of the leaves of the *R. stricta* plant have significant inhibitory effects on seed germination and seedling growth of the tested parasite weed species compared to those of control treatment (distilled water) suggesting the availability of inhibitory chemicals in the leaves of *R. stricta* to control the Cuscuta weed. It is suggested to test the negative effects of *R. stricta* leaf and stem extracts, after decomposition, on other weeds and adjacent and sensitive plants to *R. stricta*.

INTRODUCTION

Weeds are uneconomic plants that reduce crop yield (Mukhtar et al. 2012). In the new weed management methods, a detailed understanding of the dynamic relationship between weeds and crops is essential (Datta et al., 2007). Among weeds, parasitic plants have more profound effects on host plants. They take all or part of the water, carbon, and nutrients they need through the vascular tissue from the root or shoot of their host. Therefore, they are considered among the most important factors that reduce crop yield (Press and Phoenix, 2005). Parasitic plants include a diverse group of plants. Among them, the Cuscuta weed is an obligate parasite of many plants in various families with a global spread (Sandler, 2010).

Cuscuta is an annual obligate parasite from the Cuscutaceae family and *Cuscuta* genus that infects many crops especially broadleaf crops), ornamentals, native plants, and weeds worldwide (Córdoba et al., 2021; Lanini et al., 2010). Cuscuta also parasitizes trees and shrubs (Lanini et al., 2010). There are more than 150 types of Cuscuta worldwide (Ashigh and Marquez, 2010). Cuscuta seedlings emerge from the seed coat with a cotyledon-shaped hypocotyl without cotyledons. These seedlings use stomatal movements and chemical orientation to position themselves and wrap around the crop stem (Kaštier et al., 2018, Fernández-Aparicio et al., 2020). After Cuscuta wraps around the crop stem, epidermal cells are formed at the junction of disc-like meristems and form the haustorium (Vaughn, 2002). *C. campestris* Yunck is a parasite of a wide range of herbaceous plants that their seedlings do not contain chlorophyll or have a small amount in them. Instead of a regular root system, these seedlings have only a few millimeters of primary roots (Kaštier et al., 2018). Therefore, they are incapable of autotrophic growth and become old and die within 7 to 10 days after germination in the absence of host infection (Fernández-Aparicio et al., 2022). When Cuscuta attaches to the crop stem, there is no selective and effective control method against Cuscuta to protect most affected crops (Córdoba et al., 2021).

This weed lowers the growth and yield of many agricultural and horticultural plants (Lanini et al., 2010; Vaughn, 2002). During a weed survey (Sin et al., 2020), *C. campestris* was found attached to 23 weed species from 15 families. A higher frequency of parasitism of *C. campestris* was determined in *Polygonum aviculare L.*, *Amaranthus retroflexus L.*, *Chenopodium album L.*, *Ecballium elaterium (L.) A. Rich.*, *Lactuca serriola L.*,

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Portulaca oleracea L. and *Cichorium intybus L.* weed species. The infection intensity was the highest in *Lactuca serriola L.*, *Convolvulus arvensis L.*, *Portulaca oleracea L.*, *Tribulus terrestris L.*, *Ecballium elaterium (L.) A. Rich.*, *Rumex crispus L.* and *Polygonum aviculare L.* (Sin et al., 2020).

Several strategies have been proposed to control this parasitic plant, including mechanical methods (removal or deep plowing), chemical methods (use of herbicides), and biological control (biological herbicides and microbially and genetically modified plants) (Westwood et al., 2018).

In addition to the advantages of the biological control method, this method is effective in parasitic plants because of the close connection between the host and the parasitic plant. It has been explained that chemical control of these weeds is difficult, and few herbicides can selectively kill the pest plant without harming the host (Sauerbon et al., 2007).

Allelopathy is the direct effect of one plant on other plants through the release of chemicals (Garima and Devi., 2017). It is a natural tool to increase crop yield through weed control and reduce the use of chemical herbicides (Alaesaboopathi, 2010). In most plants, allelopathy causes the release of environmental chemicals from their aerial and underground parts. Allelopathic chemicals near other plants or weeds may increase or lower their specific properties. Allelopathic plants are agronomically significant and can manage weeds (Anwar et al., 2016). Allelopathic relationships between plants can be intraspecific or interspecific (Garima and Devi., 2017). In this respect, allelopathic compounds secreted by higher plants are often secondary metabolites. It has been shown that phenolics, quinones, cyanogenic glycosides, organic acids, lactones, and terpenes are chemical compounds to active allelopathy (Qureshi and Arshad, 2017). Terpenes and phenolic acids were also reported by Shankar et al., (2009) to be common types of allelopathic substances. These substances disrupt physiological processes, including photosynthesis, respiration, water, and hormone balance of receiving plants, by inhibiting enzyme activity (Soltys et al., 2013). It has been indicated that the integration of cultural control (the use of wheat stubble mulch at the beginning of the chickpea growth period) with chemical control could be suitable option for dodder management in the chickpea fields (Shamsi et al., 2018).

The Eshvarak plant, with the scientific name *Rhazya stricta* Decne, is an allelopathic evergreen shrub originating from the Karak region in Pakistan (Khan et al., 2016). This plant competes with other plants in terms of nutrients and ultimately prevents healthy growth. Also, it reduces their performance qualitatively and quantitatively. Research has shown the presence of alkaloids, glycosides, triterpenes, tannins, and aromatic substances in the leaves of this plant (Alqarawi et al., 2018).

Weeds have the greatest economic impact among all the biotic stresses that negatively affect crop yield (Pimental et al., 2005). In this respect, parasitic weeds are among the most destructive types of weeds that are difficult to control regarding their ability to extract nutrients and water from the crop and their communication with the vascular system of the product (Fernández-Aparicio et al., 2016).

As *C. campestris* is an obligate parasite of higher plants, the present study aimed to investigate the allelopathic potential of the extracts of Eshvarak (*R.* Stricta) leaves on seed germination and the growth of Cuscuta seedlings that naturally grow with it.

MATERIALS and METHODS

The *R. stricta* plant with herbarium code SH1128256.09FU was collected from Iranshhar-Khash farms (Fig. 1A). Leaves were isolated and washed entirely with water and dried in the open air under natural conditions. Leaf samples (Fig. 1B) were powdered and stored in plastic bottles at room temperature.

Fig. 1. (A) The *Rhazya stricta* plant collected from Iranshahr-Khash farms. (B) Leaf samples

Preparation of the extracts

As discussed by Khan et al., (2011), about 10 g of powdered leaves were soaked in 100 mL of sterile distilled water for 24 h at room temperature. The aqueous extract was filtered, and the final volume was adjusted to 100 mL. This extract was considered as a stock solution of aqueous extract. Other extracts contained hydroalcoholic, acetone, methanolic, and ethanolic from the *R. stricta* plant (Fig. 2). Maceration (soaking) method was used for the extraction of these extracts. For this purpose, about 10 g of powdered leaves were soaked in 70 mL of 70% alcohol plus 30 mL of sterile distilled water (to prepare the hydroalcoholic extract), 100 mL of acetone (to prepare the acetone extract), 100 mL of methanol (to prepare methanolic extract), and 100 mL of ethanol (to prepare methanolic extract). Next, the extracts were kept for 72 h at room temperature and on a shaker. After passing the desired time, the extracts were smooth, and the solvents were concentrated at a temperature of less than 40 °C by a rotary device. Finally, after drying by an incubator device (temperature of 37 °C), the residue of each extract was kept at 4 °C and used to perform further experiments in sterile Petri dishes.

Treatments and experimental design

The treatments contained methanolic, hydroalcoholic, ethanolic, aqueous, and acetonic extracts from *R. stricta* plant. The control treatment included distilled water. Cuscuta seeds were disinfected with 2% sodium hypochlorite solution for 15 min. A few sterile filter papers (Whatman No. 1) were placed in each sterilized 9 cm Petri dish the seeds were placed on them The seeds in each Petri dish were moistened with 10 mL of each prepared extract and allowed to germinate. The experiment was conducted in the form of a completely randomized design in three replications. Fifty seeds were used in each replication. The petri dishes were kept at a constant temperature (25 ± 1°C) with a 16 h/8 h (light/dark) cycle for 7 days. During these tests, no fungal contaminations were detected.

Germination indices determination

After 7 days, the germination percentage, germination rate, and mean germination time were determined. All seedlings were regarded as having germinated if their radicles measured at least 2 mm in length. When the hypocotyls rose above the surface of the growth medium, seedling emergence was recorded. The number of germinated seeds was counted and recorded daily for up to 7 days. The final count was performed after 7 days, and the final germination percentage was calculated using Equation 1 indicated in Table 1. The mean germination time (MGT) was calculated as a measure of the speed of germination or emergence using Equation 3 indicated in Table 1. Also, germination vigor was calculated based on measuring the length of roots and stems using Equation 4 indicated in Table 1. On the last day of the experiment, 10 samples were randomly taken from each petri dish to measure the length of roots and stems. Afterward, the aerial part and the root were separated and their lengths were measured. A ruler was used to measure plant height and root length and the results were stated in centimeters.

Statistical analysis

The data obtained with the complete random design were analyzed using SPSS ver.16 software. Also, a comparison of means was performed using Duncan's multiple-range test.

RESULTS

The analysis of variance (ANOVA) results of the effect of different extracts of *R. stricta* plant on the characteristics of germination and growth of Cuscuta seedlings showed that *R. stricta* extract had a significant effect on all the measured characteristics (Table 2). The effects of different extracts of leaves of *R. stricta* on seed germination of Cuscuta are shown in Table 3. The results of this study showed that all extracts significantly decreased the germination percentage of Cuscuta seeds than in control. The control treatment (distilled water) had the highest percentage of germination (98.33%) and the lowest percentage of seed germination was seen in the aqueous extract of the *R. stricta* plant (8.66%). In this respect, the most reduction in the number of germinated seeds was related to aqueous extract of the leaves of *R. stricta*, which showed significant inhibitory effects on seed germination and seedling growth of the tested weed. This finding indicated the availability of inhibitory chemicals in leaf of *R. stricta* (Table 3). Also, germination vigor (0.17) and germination rate (0.61) of Cuscuta seeds decreased using the aqueous extract of *R. stricta* compared with other treatments (Table 3 and Fig. 3).

The mean germination time (MGT) of Cuscuta seeds was significantly ($P < 0.05$, Table 2) affected by extracts of leaves of *R. stricta*. Mean germination time was observed at 2.10-4 days in various extracts of the leaves of the *R. stricta* plant. The longest (4 days) delay in mean germination time was observed when Cuscuta seeds were treated with the aqueous extract of leaves of *R. stricta*, whereas the lowest mean germination time reduction (2.10 days) was found in the control treatment (distilled water, Table 3).

The rootlet length and plantlet length of Cuscuta seeds were significantly ($P < 0.05$, Table 2) affected by various extracts of leaves of *R. stricta*. When Cuscuta seedlings were treated with aqueous extract of *R. stricta*, the maximum reduction in the shoot length (0.29 cm) and root length (0.19 cm) was observed compared to those of the control treatment (Table 3).

Table 1. Equations of determining germination indices

Equation number	Index	Equation	References
1	Germination percentage (GP)	$GP = \frac{n}{N} \times 100$	Panwar and Bhardwaj 2005
2	Germination rate (GR)	$GR = \sum \frac{n_i}{t_i}$	Kulkarni et al., 2007
3	Mean germination time (MGT)	$MGT = \frac{\sum (t_i \times n_i)}{\sum n}$	Kulkarni et al., 2007
4	Germination vigour (GV)	$GV = \frac{GR \times Mean(PL + RL)}{100}$	ISTA 2009

n = total of germinated seeds during period, n_i= The number of germinated seeds at an interval of distinct period, t_i= The number of days after the start of germination, N = Number of used seeds, ISTA: International Seed Testing Association, PL: Plumle length (Plantlet), RL: Radicle length (Rootlet).

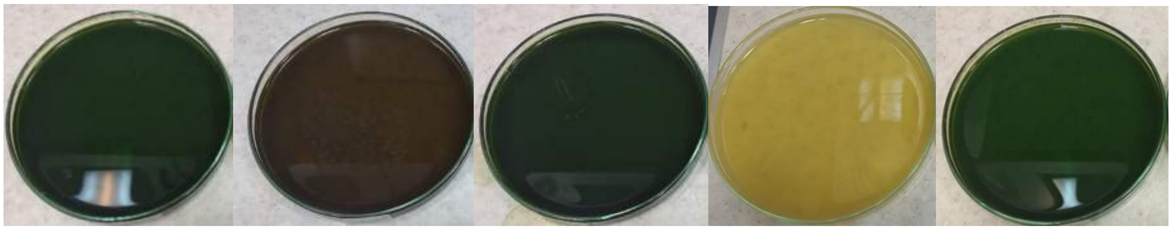

Fig. 2. From left to right, the methanolic, hydro alcoholic, ethanolic, aqueous, and acetonic extracts from *Rhazya stricta* plant, respectively.

Table 2. ANOVA data (mean squares) for the effect of different extracts of R. stricta plant on the seed germination of Cuscuta weed

Source of Variation	df	Mean squares					
		Germination percent	Germination vigour	Germination rate	Mean germination time	Rootlet lenght	Plantlet lenght
Treatments	5	3298.456**	1079.689**	17.30**	1.658**	5.182**	5.215**
Error	12	10.94	4.38	0.05	0.010	0.019	0.013
Total	17	--	--	--	--	--	--

** *:significant at $P \leq 0.01$

Table 3. Mean comparison of effects of different extracts of *R. stricta* plant on the seed germination of cuscuta weed

Treatment	Germination percent (%)*	Germination vigor*	Germination rate*	Mean germination time* (days)	Rootlet length (cm)*	Plantlet length (cm)*
Control (distilled water)	98.33a	47.00a	11.7a	2.10d	3.65a	3.8a
Hydroalcoholic extract	47.33b	0.95b	3.37b	3.80b	0.80b	1.10b
Etanolic extract	40.00c	0.80b	285c	3.31c	0.50c	0.70c
Acetonic extract	28.66d	0.58b	2.04d	3.76b	0.70bc	1.00b
Methanolic extract	9.33e	0.18b	0.66e	4.10a	0.24d	0.32d
Aqueous extract	8.66e	0.17b	0.61e	4.00a	0.19d	0.29d

*Means followed by the same letters in each column are not significant at 1% probability.

Fig. 3. Illustration of the germination of Cuscuta seeds in Petri dishes containing extracts of *R. stricta*; from left to right: Hydroalacolic extract; Ethanolic extract; Aqueous extract and distilled water (control treatment), respectively.

DISCUSSION

The present study showed the allelopathic effects of the *R. stricta* plant on the germination and growth of Cuscuta seeds from the initial screening. Based on the results, aqueous leaf extracts had the strongest allelopathic effect on seed germination. Another research (Khan Khattak et al., 2015) has shown the effectiveness of the extracts of *R. stricta* plant on seed germination and seedling growth of *Pennisetum typhoides* suggests that they may act as a source of allelochemicals after being released into the soil or after decomposition, thereby negatively affecting nearby plants.

The results of the current study indicated that the mean germination time of the Cuscuta seeds was 2 days under control treatment and increased to 4 days after applying an aqueous extract of leaves of *R. stricta*. An increase in the mean germination time of the Cuscuta seeds indicated that the treatment of these seeds with *R. stricta* leaf extract is delaying their germination. Also, Lalbiakdika *et al*. (2022) showed that the allelopathic inhibitory effects of the leaf extracts of tested weed species on germination and seedling growth of rice were stronger than the effects of the extracts of other weed parts demonstrating that the leaves of tested weeds produce more water-soluble phytotoxins which can slow down germination of rice seeds to a greater extent (Lalbiakdika *et al*. 2022). It has also been shown that the plants release phytochemicals from dead tissues, and their incorporation into the soil can be accelerated by leaching. As a result, it intensifies their harmful effects in the field (Inderjit and Duke, 2003). It seems that this aspect significantly inhibited Cuscuta weed when the leaf extracts of the *R. stricta* plant were used in this experiment.

The effectiveness of the *R. stricta* plant extracts on the germination and growth of Cuscuta seeds shows that the leaves of this plant can act as a source of allelochemicals after decomposition, thereby negatively affecting adjacent or successive plants. Various phytotoxicities observed from the *R. stricta* plant can be attributed to the presence of variable amounts of phytotoxic substances in different parts of the plant washed under natural conditions. Some recent studies have shown the phytotoxic/allelopathic effects of aqueous extracts of weeds, including Congress grass (*Parthenium hysterophorus)* (Singh et al. 2003) on radish and chickpea, and Billygoat weed *(Ageratum conyzoides*) and *Cleome viscosa* on sesame (Anbarasan and Prabhakaran, 2015).

The measurement of seedling root and shoot lengths has been reported to be the most commonly used characteristic to illustrate allelopathic potential. (Khaliq et al. 2013). When different extracts of leaves of *R. stricta* were applied to Cuscuta seedlings, the results of effects of *R. stricta* plant extracts on growth control of Cuscuta seedlings were significantly different. The findings suggested that the aqueous extract of *R. stricta* had the highest significant impact on the development and growth of Cuscuta seedlings compared to other treatments and the control treatment. Similarly, the rootlet length and plantlet length were affected by *R. stricta* extracts, with the most reduction values related to aqueous extract (i.e., 0.19 cm in rootlet length and 0.29 cm in plantlet length). Also, another study strongly demonstrated the release of phototoxic chemicals during the preparation of aqueous extracts (Abdul Raoof and Siddiqui, 2012). It has been shown that the allelopathic effects of some multipurpose tree species significantly affect seed germination and seedling growth of wheat and some related weeds (Khanh et al., 2004). Overall, the results of this study and similar studies such as Khan Khattak et al., (2015) showed that the leaf extract of poisonous plants such as *R. stricta* reduced the growth of some weeds.

CONCLUSIONS

The aqueous extract of the leaves of the *R. stricta* plant showed significant inhibitory effects on seed germination and seedling growth of the Cuscuta weed. This finding indicates the availability of inhibitory chemicals in leaf extracts of *R. stricta*.

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اثرات بالقوه آللوپاتی عصاره گیاه اشورک (*Rhazya stricta*) بر کنتــرل رشد گیاهچه های علف هرز سس (*Cuscuta campestris*)

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خاصیت آللوپاتی

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چکیده - در بین علف‌های هرز، گیاهان انگلی اثرات بیشتری بر روی گیاهان میزبان دارند. گیاه سس (*Cuscuta campestris*) به‌دلیل پراکندگی جغرافیایی وسیع، دامنه میزبانی بالا و روش‌های مدیریتی ناکارآمد، به یکی از آسیب‌زاترین گیاهان انگلی تبدیل شده است. پژوهش حاضر با هدف کنترل بیولوژیک علف هرز سس در مزرعه طراحی شد. به همین منظور، آزمایشی برای بررسی اثرات آللوپاتیک عصاره گیاه اشورک (*Rhazya Stricta*) بر کنترل جمعیت این علف هرز در قالب یک طرح کاملا تصادفی با سه تکرار در آزمایشگاه دانشگاه ولایت ایرانشهر انجام شد. در این آزمایش، اثرات آللوپاتی منفی عصاره‌های مختلف گیاه اشورک شامل عصاره‌های استونی، آبی، متانولی، اتانولی و هیدروالکلی بر روی سس در مقایسه با آب مقطر (بعنوان تیمار شاهد) مورد بررسی قرار گرفت. نتایج نشان دادند که عصاره آبی گیاه آشورک در مقایسه با سایر تیمارها، درصد جوانه زنی ، قدرت جوانه‌زنی، سرعت جوانه زنی ، طول ریشه و طول ساقه سس را به‌طور معنی‌داری در مقایسه با سایر تیمارها کاهش داد. همچنین، عصاره های آزمایش شده برگ گیاه اشورک اثرات بازدارندگی قابل توجهی بر جوانه‌زنی بذر و رشد گیاهچه های گونه علف هرز انگل مورد آزمایش در مقایسه با تیمارها شاهد (آب مقطر)داشت، که نشان دهنده در دسترس‌بودن مواد شیمیایی بازدارنده در برگ و ساقه گیاه اشورک برای کنترل علف های هرز سس می باشد. پیشنهاد می شود اثرات منفی عصاره برگ و ساقه گیاه اشورک، پس از تجزیه، بر روی سایر علف های هرز و گیاهان مجاور و حساس به گیاه اشورک مورد آزمایش فرار گیرد.