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SPECIAL TOPICS

RESEARCH ARTICLE

Climate change, an emerging threat to weed management

Assessment of allelopathic influence of some cruciferous species on germination indicators of field dodder seeds

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Abstract: Background: Field dodder is a parasitic plant that has long been a concern in agriculture and natural ecosystems and a difficult plant to control. **Objective:** This study aimed to evaluate the allelopathic effect of four species of cruciferous plants on germination parameters of field dodder seeds and to determine the isothiocyanate compounds in these plants. **Methods:** The experiment was implemented in two trails during 2020 and 2021 using a completely randomized design. Cruciferous plant extracts were prepared at concentrations of 2%, 6%, 10%, and 20%. For each petri dish populated with field dodder seeds 5 ml of cruciferous plant extract was added based on the experiment design while 5 ml of distilled water was added to control plots. The germination indicators were monitored, and the samples of cruciferous plants were prepared to determine their contents of ITCs. **Results:** The effect of extracts on germination indicators was weak at concentrations of 2 and 6%. The effect increased when the concentration increased to 10%. At a 20% concentration of turnip extract, field dodder seed germination decreased significantly to 2.1%. Arugula, broccoli, and black radish extracts at 20% concentration had germination percentages ranging from 9.6% to 12.5%. Other indicators significantly decreased compared to the control plots. GC-MS analysis showed the highest ITC compounds percentage in turnip (56.6%) and the lowest in black radish (29.2%). **Conclusions:** Cruciferous plants can be used as an alternative control method against field dodder by mixing these plants into the soil to reduce the soil's stock of field dodder seeds.

Keywords: Field dodder; Isothiocyanate; Allelopathic; Germination indicators; Cruciferous plants; GC-MS

1. Introduction

Field dodder, scientifically known as *Cuscuta campestris* Yunck., is a parasitic plant belonging to the Convolvulaceae family. Field dodder lacks chlorophyll and relies entirely on other host plants for nutrients and water, making it a significant threat to crops cultivation (Costea, Stevanovic, 2010). Field dodder adaptability and ability to infest multiple plant species make it a challenging weed to control in agricultural settings, emphasizing the need for effective management strategies to minimize its impact on crop production (Nadler-Hassar, Rubin, 2003; Ustuner, 2018).

It was reported that yield losses caused by field dodder in some crops belonging to the Solanaceae family (such as eggplant, tomato, potato, and pepper) ranged between 50–90% (Nadler Hassar, Rubin, 2003; Lian et al., 2006; Ustuner, 2020). As well as it was reported that yield loss caused by field dodder in alfalfa was 57% (Dawson et al., 1994), 30% in carrot (Konieczka et al., 2009), 86% in chickpeas and 87% in lentils (Mishra, 2009).

Several studies have reported about the potent allelopathic properties of cruciferous plants, specifically those belonging to the *Brassica* genus. These plants release isothiocyanates, chemical compounds known for their toxicity to soil-borne pathogens, nematodes, fungi, and weeds (Uremis et al., 2009; Jabran et al., 2015; Cipollini, 2016). It was reported that arugula extract at a concentration of 2% under laboratory conditions reduced the germination of *Sonchus oleracens* by 70% and *Sorghum halepense* by 62.5% (Shaker et al., 2010). In one study, the allelopathic effect of five species of cruciferous plants, namely white cabbage, red cabbage, broccoli, turnip, and arugula, was tested at concentrations of 2%, 5%, 10%, and 20% on the germination of *S. halepense* seeds. The results showed that arugula extract at concentrations 10 and 20% prevented the germination by 100% (Elsekran et al., 2023). The allelopathic effect of cruciferous plants in their biosphere is attributed to isothiocyanate compounds, which are considered one of the most important hydrolysis products of glucosinolates (GLSs) (Wittstock, Halkier, 2002).

GLSs are chemically inert compounds subject to hydrolysis by the enzyme myrosinase, also known as beta-thioglucosidase, resulting in the formation of biologically active chemical compounds. The specific hydrolysis products of GLSs exhibit variability contingent upon the prevailing degradation conditions (Bones,

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Rossiter, 1996). Enzymatic action catalyzes the liberation of glucose and the formation of an unstable compound known as aglucon-thiohydroxymate-O-sulfate. The conversion of aglucon into various classes of degradation compounds, including isothiocyanates, thiocyanates, nitriles, epithionitriles, hydroxynitriles, oxazolidine-2-thiones, or indoles, is contingent upon factors such as the structure of the hydrolyzed GLSs, pH, temperature, the presence of Fe^2+ ions, and the presence of additional protein agents within the hydrolysis environment (Wittstock, Halkier, 2002). Isothiocyanate (ITC) compounds represent significant hydrolysis by products derived from GSLs, exhibiting notable biochemical reactivity. ITCs exhibit variability in their chemical properties and taxonomic classification, yet they share a distinctive structural motif characterized by the presence of a carbon-nitrogen-sulfur double bond (Martins et al., 2004).

The primary objective of this study was to assess the allelopathic effects of turnip, arugula, broccoli, and black radish on germination indicators of field dodder seeds. Furthermore, the study aimed to determine the content of isothiocyanates in these cruciferous plants.

2. Material and Methods

The experiment was implemented in two trails during 2020 and 2021 using a completely randomized design in Faculty of Agriculture laboratories at Kahramanmaras Sutcu Imam University, Turkey. Cruciferous plants were also cultivated at the trial area of the East Mediterranean Transitional Zone Agricultural Research Institute in Kahramanmaras (37.53564°N, 36.91800°E) to obtain samples used for the laboratory work.

2.1 Preparation of plant materials

The field dodder seeds used in the experiment were collected in 2019 and 2020 seasons from eggplant fields infected with field dodder located in the Kahramanmaras province in Turkey.

Broccoli seedlings were cultivated in the field on Dec 18th, 2019, and on Dec 20th, 2020. Turnip, arugula, and black radish seeds were sown on Feb 10th, 2020, and on Feb 11th, 2021. To obtain healthy plants with a strong vegetative, flowering and root system, the necessary agricultural practices were provided to the plants accurately in the field. NPK 20-20-20 fertilizer was applied in three separate batches based on the specific nutritional requirements of the plants. Irrigation was carried out at 10-day intervals, considering the natural rainfall patterns. Additionally, four rounds of hand hoeing were performed to control weeds. As a precaution against fungi and insects, a fungicide (ridomil) and an insecticide (thiamethoxam) were applied. For both years of experiment, the cruciferous plants were sampled during the head formation stage of broccoli and the flowering stage of turnip, arugula, and black radish by collecting the entire plants, including the roots, stem, leaves, and flowering and fruiting parts. These samples were cleaned and dried under room conditions (25 ± 2 °C). After the samples were completely dried, they were ground separately using a coffee grinder, and the powders were placed in plastic bags and kept at room temperature. Table 1 shows the species and varieties of cruciferous plants used in this experiment.

2.2 Preparation of cruciferous plant extracts

For the laboratory experiment, from each sample of cruciferous plant powders, weights of 2, 6, 10, and 20 g were taken, and each unit of weight was mixed with 100 ml of distilled water to obtain concentrations of 2, 6, 10, and 20%, respectively. The prepared extracts were placed in a shaker at 200 rpm and 25 ± 2 °C for 8 hours. Then the mixtures were filtered using Whitman filter paper to separate all impurities.

Cruciferous plant samples were prepared for GC-MS analysis according to Vaughn and Berhow (2005) method in the Plant Protection Department laboratory, at Kahramanmaras Sutcu Imam University. To prepare the samples, an analytical balance was used to weigh ten grams of powder from each cruciferous plant. The powder samples were subjected to defatting for 24 hours using a Soxhlet extractor with hexane. Subsequently, the plant powders were dried thoroughly in a fume hood until complete dryness. The defatted powders were combined with 30 ml of potassium phosphate (0.05M) buffer in separate flasks. To each mixture, 50 ml of dichloromethane was added, and the flasks were placed in a shaker under conditions of 25 °C and 200 rpm for 8 hours. Thereafter, 10 g of NaCl and 10 g of Na_2SO_4 were added and thoroughly mixed. The mixtures were filtered using filter paper, with additional dichloromethane added during the filtration process. The resulting dichloromethane solutions were transferred to ampoules and labeled with corresponding numbers based on the respective plants. The ampoules were then sent to the chemistry department of Ataturk University for GC-MS analysis.

Table 1. Species and varieties of cruciferous plants used in the experiment

Plant species	Scientific name	Plant varieties
Turnip	*Brassica rapa* L. subsp. *rapa*	Kahramanmaras Salgam
Arugula	*Eruca vesicaria* subsps. *sativa* Mill.	Istanbul Rokasi
Broccoli	*Brassica oleracea* L. var. *italica*	Sakura F1
Black radish	*Raphanus sativus* L. var. *niger* J. Kern	Siyah Inci

2.3 Experiment methodology

The field dodder seed dormancy was broken by placing seeds in concentrated sulfuric acid (98%) for 5 minutes, then washing the seeds with running water for 10 minutes (Almhemed et al., 2020). Petri dishes were prepared by sterilizing them with methanol (95%), then a double layers of Whitman filter paper were placed inside each petri dish. In each petri dish 30 seeds of field dodder were populated. To each petri dish 5 ml of cruciferous plant extract was added based on the experiment design while 5 ml of distilled water was added to the control plots. Petri dishes were covered with stretch film and placed in an incubator chamber at 28 °C with a 12:12 light-dark cycle.

2.4 Germination rate of field dodder seeds (G%)

The seed germination rate was recorded on a daily basis from the beginning to the end of the experiment, the percentage of germination was calculated using the formula (1).

$$G\% = (\text{number of germinated seeds} / \text{total number of seeds}) \times 100 \tag{1}$$

2.5 The length of field dodder shoots (L)

For each germinated seed, the shoot length of field dodder was measured three days after germination using a small ruler to the nearest mm.

2.6 Speed of field dodder seed germination (SG)

The number of germinated seeds was recorded on a daily basis and the germination speed of field dodder seeds was calculated using the formula (2) proposed by Czabator (1962).

$$SG = (X1/Y1) + (X2/Y2) + \ldots + (Xn/Yn) \tag{2}$$

Where, X1: The number of germinated seeds in the first day, Y: Number of days, Xn: The number of germinated seeds in last day.

2.7 Mean germination time (MGT)

The mean germination time of field dodder seeds was calculated using formula (3) proposed by Ellis and Roberts (1981).

$$MGT = (X1 \times D1) + (X2 \times D2) + (X3 \times D3) + \ldots / Y\ (\text{Total days number}) \tag{3}$$

Where, X: The number of germinated seeds, D: specific day, Y: Number of days.

2.8 Mean daily germination (MDG)

The mean daily germination of field dodder seeds was calculated using formula (4) proposed by Czabator (1962).

$$MDG = \text{The total number of germinated seeds} / \text{The total number of days} \tag{4}$$

2.9 Peak value (PV)

The peak value was calculated using formula (5) proposed by Czabator (1962).

$$PV = Xh/Yh \tag{5}$$

Where, Xh: The highest number of germinated seeds, Yh: The number of days.

2.10 Germination value (GV)

The germination value was calculated using formula (6) proposed by Czabator (1962), according to the peak value and mean daily germination.

$$GV = PV/MDG \tag{6}$$

2.11 Data analysis

The experiment was conducted in two trails during 2020 and 2021. Independent T-test was used to compare the means between the first and second years. Based on T-test results, the hypothesis that no significant differences in means between the two years was accepted. Ultimately, the average of the two years was used in the results discussion. The data collected were subjected to ANOVA analysis through MSTAT-C software (Version 2.10). The Least Significant Difference (LSD) test at a significance level of 0.01 was utilised to compare the means.

3. Results

3.1 Effect of cruciferous plant extracts at a concentration of 2% on germination indicators of field dodder seeds

The germination rate of field dodder seeds ranged between a maximum of 49.6% in control plots and a minimum of 42.9% in turnip treatment at a concentration of 2%. It was observed that the germination rate of field dodder seed treated with 2% of turnip and arugula extracts was significantly lower than the control plots. There were no significant differences between the treatments of turnip, arugula, and broccoli as the recorded germination rates were 42.9%, 43.8%, and 46.3% for these three treatments, respectively. There were no significant differences between the treatment of black radish and the control plots as the germination rate was 48.3% and 49.6%, respectively. The field dodder shoot length was not significantly affected by the different treatments as it ranged between 5.04 cm and 5.16 cm for all treatments, including control plots. Similarly, the speed of germination was not significantly affected by the different treatments as it ranged between 2.41 and 2.68 for all treatments, including control plots. The lowest mean germination time for field dodder seeds was recorded in turnip treatment at a concentration of 2% with 7.80, which significantly outperformed black radish treatment and control plots that recorded 9.28 and 9.15, respectively. Conversely, the treatment of turnip at a concentration of 2% did not differ significantly with arugula and broccoli treatments that recorded 8.15 and 8.58, respectively. No significant differences were recorded in terms of mean daily germination in the treatments of turnip, arugula, and broccoli at concentrations of 2% with values of 1.29, 1.31, and 1.39 for these three treatments, respectively. Meanwhile, no significant differences were observed between the black radish treatment at a concentration of 2% and the control plot as the mean daily germination recorded 1.45, and 1.50, respectively. The peak value ranged between a minimum of 0.23 in the treatment of arugula and a maximum of 0.30 in the treatment of black radish at a concentration of 2% and control plots. No significant differences were observed between treatment with the arugula extract at a concentration of 2% and treatment with the turnip extract at the same concentration, which recorded a peak value of 0.24. In contrast, the treatment with the arugula extract at a concentration of 2%, significantly outperformed the treatment with the broccoli extract at the same concentration, which recorded a peak value of 0.31. The two treatments with turnip and arugula extracts at a concentration of 2% outperformed other treatments in reducing the germination value, which recorded 0.31 and 0.30 for these two treatments, respectively. While there were no significant differences between the treatments of broccoli and black radish at a concentration of 2% and control plots as these three treatments recorded a germination value of 0.44, 0.44, and 0.49, respectively (Table 2).

3.2 Effect of cruciferous plant extracts at a concentration of 6% on germination indicators of field dodder seeds

All treatments with cruciferous plant extracts at a concentration of 6% reduced the germination rate of field dodder seeds compared to the control plots. The two treatments with turnip and arugula extracts outperformed the treatment with black radish extract, as the germination rate in these three treatments was 31.3%, 32.5%, and 38.8%, respectively. The treatment with turnip extract at a concentration of 6% significantly outperformed other treatments in terms of reducing the length of field dodder shoot, which recorded 4.08 cm. However, no significant differences were observed between the treatments with arugula, broccoli, and black radish extracts at 6% concentrations, as the length of field dodder shoot ranged between 4.58 cm and 4.85 cm in these three treatments. As well, all treatments with cruciferous plant extracts at a concentration of 6% reduced the speed germination of field dodder seeds compared to the control plots. The treatment with turnip extract at a concentration of 6% outperformed the black radish treatment at the same concentration in terms of reducing the speed germination of seed, which recorded 1.65, but it was statistically similar to the treatments with arugula and broccoli extracts that recorded 1.84 and 1.97, respectively. The mean germination time for treatments with extracts of turnip, arugula, broccoli, and black radish at a concentration of 6% ranged between 5.91 and 7.24, as all these treatments reduced the mean germination time compared to control plots. Similarly, the mean daily germination for treatments with extracts of turnip, arugula, and broccoli at a concentration of 6% ranged between 0.94 and 1.09, as these three treatments reduced the mean daily germination compared to control plots. The peak value of 0.20 was recorded in the two treatments of turnip and arugula at a concentration of 6%, which significantly outperformed the control plots, but were statistically similar to the treatments of broccoli and black radish at a concentration of 6% that recorded peak value of 0.26 for both treatments. All treatments with cruciferous plant extracts at a concentration of 6% reduced the germination value of field dodder seeds compared to the control plots. The treatment with turnip extract at a concentration of 6% reduced the germination value to 0.20, which significantly outperformed the treatments of broccoli and black radish at a concentration of 6% that recorded germination values of 0.29 and 0.31, respectively, but it was statistically similar to the treatment of arugula which recorded germination value of 0.20 (Table 3).

Table 2. Effect of cruciferous plant (turnip, arugula, broccoli, and black radish) extracts at a concentration of 2% on germination indicators of field dodder seeds

Treatments	G%	L/cm	SG	MGT	MDG	PV	GV
Ctrl	49.6^{c}	5.16^{a}	2.68^{a}	9.15^{bc}	1.50^{b}	0.30^{bc}	0.49^{b}
Turnip 2%	42.9^{a}	5.10^{a}	2.44^{a}	7.80^{a}	1.29^{a}	0.24^{ab}	0.31^{a}
Arugula 2%	43.8^{ab}	5.16^{a}	2.41^{a}	8.15^{ab}	1.31^{a}	0.23^{a}	0.30^{a}
Broccoli 2%	46.3^{abc}	5.12^{a}	2.50^{a}	8.58^{abc}	1.39^{ab}	0.31^{c}	0.44^{b}
Black radish 2%	48.3^{bc}	5.04^{a}	2.50^{a}	9.28^{c}	1.45^{b}	0.30^{bc}	0.44^{b}
LSD 0.01	4.645	0.289	0.297	1.122	0.136	0.068	0.096

Values followed by the same letter(s) in the same column are not significantly different from each other at 0.01 level of probability, (G: field dodder seeds germination, L: the length of field dodder shoots, SG: speed of germination, MGT: mean germination time, MDG: mean daily germination, PV: peak value, GV: germination value).

3.3 Effect of cruciferous plant extracts at a concentration of 10% on germination indicators of field dodder seeds

All treatments with cruciferous plant extracts at a concentration of 10% reduced the germination rate of field dodder seeds compared to the control plots. The turnip treatment at a concentration of 10% achieved the lowest germination rate for field dodder seeds by 9.6%, as it significantly outperformed the black radish treatment which recorded germination rate of 14.5% and statistically did not differ from arugula and broccoli treatments at a concentration of 10% that recorded germination rate of 11.3% and 11.7%, respectively. All treatments with cruciferous plant extracts at a concentration of 10% led to a decrease in the length of field dodder shoots compared to the control plots. The treatments of turnip and arugula at a concentration of 10%, significantly outperformed the treatments of broccoli and black radish in terms of reducing the length of field dodder shoot, as it recorded 2.37, 2.51, 3.10, and 3.28 cm for these four treatments, respectively. No significant differences were observed between all treatments in terms of germination speed of field dodder seeds, peak value, and germination value, which ranged between 0.54–0.71, 0.10–0.16, and 0.03–0.07 for these three indicators, respectively. However, all treatments significantly outperformed the control plots in terms of reducing speed of seed germination, peak value, and germination value. The turnip treatment at a concentration of 10% achieved the lowest mean germination time of 1.71 and the lowest mean daily germination of 0.29, as this treatment significantly outperformed the treatment of black radish at a concentration of 10%, which recorded mean germination time of 2.88 and mean daily germination of 0.44. The turnip treatment at a concentration of 10% statistically similar to the treatments of arugula and broccoli at a concentration of 10%, that recorded mean germination time of 2.03 and 1.83 and mean daily germination of 0.34 and 0.35 for these two treatments, respectively (Table 4).

3.4 Effect of cruciferous plant extracts at a concentration of 20% on germination indicators of field dodder seeds

All treatments with cruciferous plant extracts at a concentration of 20% reduced the germination rate of field dodder seeds significantly compared to the control plots. The turnip treatment at a concentration of 20% significantly outperformed the treatments of arugula, broccoli, and black radish at a concentration of 20% in terms of reducing the germination rate of field dodder seeds, as it recorded 2.1%, 9.6%, 10.4%, and 12.5% for these four treatments, respectively. The treatment with turnip extract at a concentration of 20% significantly outperformed other treatments and control plots in terms of reducing the length of field dodder shoots, as it recorded 0.40 cm. Treatments with arugula, broccoli, and black radish extracts at concentrations of 20% significantly reduced the length of field dodder shoots compared to the control, as it recorded 0.76, 1.01, and 1.16 cm for these three treatments, respectively. The treatment with turnip extract

Table 3. Effect of cruciferous plant (turnip, arugula, broccoli, and black radish) extracts at a concentration of 6% on germination indicators of field dodder seeds

Treatments	G%	L/cm	SG	MGT	MDG	PV	GV
Ctrl	49.6^{c}	5.16^{c}	2.68^{c}	9.15^{c}	1.50^{c}	0.30^{b}	0.49^{d}
Turnip 6%	31.3^{a}	4.08^{a}	1.65^{a}	5.98^{ab}	0.94^{a}	0.20^{a}	0.19^{a}
Arugula 6%	32.5^{a}	4.58^{b}	1.84^{ab}	5.91^{a}	0.98^{a}	0.20^{a}	0.20^{ab}
Broccoli 6%	36.3^{ab}	4.73^{b}	1.97^{ab}	6.74^{ab}	1.09^{ab}	0.26^{ab}	0.29^{bc}
Black radish 6%	38.8^{b}	4.85^{bc}	2.06^{b}	7.24^{b}	1.16^{b}	0.26^{ab}	0.31^{c}
LSD 0.01	5.674	0.404	0.367	1.272	0.167	0.068	0.096

Values followed by the same letter(s) in the same column are not significantly different from each other at 0.01 level of probability, (G: field dodder seeds germination, L: the length of field dodder shoots, SG: speed of germination, MGT: mean germination time, MDG: mean daily germination, PV: peak value, GV: germination value).

Table 4. Effect of cruciferous plant (turnip, arugula, broccoli, and black radish) extracts at a concentration of 10% on germination indicators of field dodder seeds

Treatments	G%	L/cm	SG	MGT	MDG	PV	GV
Ctrl	49.6^{c}	5.16^{c}	2.68^{b}	9.15^{c}	1.50^{c}	0.30^{b}	0.49^{b}
Turnip 10%	9.6^{a}	2.37^{a}	0.54^{a}	1.71^{a}	0.29^{a}	0.10^{a}	0.03^{a}
Arugula 10%	11.3^{ab}	2.51^{a}	0.60^{a}	2.03^{a}	0.34^{ab}	0.10^{a}	0.04^{a}
Broccoli 10%	11.7^{ab}	3.10^{b}	0.71^{a}	1.83^{a}	0.35^{ab}	0.13^{a}	0.05^{a}
Black radish 10%	14.5^{b}	3.28^{b}	0.71^{a}	2.88^{b}	0.44^{b}	0.16^{a}	0.07^{a}
LSD 0.01	3.696	0.554	0.226	0.787	0.118	0.068	0.068

Values followed by the same letter(s) in the same column are not significantly different from each other at 0.01 level of probability, (G: field dodder seeds germination, L: the length of field dodder shoots, SG: speed of germination, MGT: mean germination time, MDG: mean daily germination, PV: peak value, GV: germination value).

at a concentration of 20% significantly outperformed other treatments and control plots in terms of reducing the germination speed of field dodder seeds, recording 0.09. No significant differences were observed among the treatments with arugula, broccoli, and black radish extracts at concentrations of 20%, as the germination speed of field dodder seeds ranged between 0.45 and 0.62 for these three treatments, which significantly outperformed control plots as well. The treatment with turnip extract at a concentration of 20% significantly outperformed other treatments and control plots in terms of reducing the mean germination time, recording 0.45. No significant differences were observed among the treatments with arugula, broccoli, and black radish extracts at concentrations of 20%, as the mean germination time ranged between 1.84 and 2.39 for these three treatments, which significantly outperformed control plots. The treatment with turnip extract at a concentration of 20% significantly outperformed other treatments and control plots in terms of reducing the mean daily germination, recording 0.06. No significant differences were observed among the treatments with arugula, broccoli, and black radish extracts at concentrations of 20%, as the mean daily germination ranged between 0.29 and 0.38 for these three treatments, which significantly outperformed control plots as well. No statistically significant differences were observed between all treatments in terms of the peak value, which ranged between 0.06 and 0.11, and in terms of the germination value, which ranged between 0.01 and 0.04, as all treatments led to a decrease in these two indicators compared to the control plots (Table 5).

3.5 Identification of isothiocyanate compounds (ITCs) in cruciferous plants

In turnip, 6 isothiocyanate (ITC) compounds have been identified; Allyl ITC, 3-Butenyl ITC, Cyclopentyl ITC, Heptyl ITC, Phenethyl ITC, and Octyl ITC. The total percentage of ITC compounds was 56.6% and the dominant ITC compound was Allyl ITC at 17.24%.

In arugula, 4 isothiocyanate compounds have been identified; benzyl ITC, phenethyl ITC, sulforaphane, and 1-naphthyl ITC. The total percentage of ITC compounds was 36.23%, while the predominant ITC compound was 1-naphthyl ITC at 13.76%.

In broccoli, 4 isothiocyanate compounds were identified; Allyl ITC, Raphasatin, Iberin, and sulforaphane. The total percentage of ITC compounds was 30.47% and the predominant ITC compound was sulforaphane at 16.27%.

In black radish, 5 isothiocyanate compounds have been identified; Allyl ITC, Isobutyl ITC, Benzyl ITC, Raphasatin, and Phenethyl ITC. The total percentage of ITC compounds was 29.2%, and Isobutyl ITC was the predominant ITC compound at 10.28% (Table 6).

Table 6. Kinds and percentage of ITC compounds in cruciferous plants (turnip, arugula, broccoli, and black radish) based on the GC-MS analysis

Cruciferous plants	Isothiocyanate compounds	Percentage (%)
Turnip	Allyl ITC	17.24
	3-Butenyl ITC	10.09
	Cyclopentyl ITC	6.35
	Heptyl ITC	12.44
	Phenethyl ITC	5.96
	Octyl ITC	4.52
	Total	56.6
Arugula	benzyl ITC	7.12
	Phenethyl ITC	4.32
	sulforaphane	11.03
	1-Naphthyl ITC	13.76
	Total	36.23
Broccoli	Allyl ITC	1.24
	Raphasatin	7.14
	Iberin	5.82
	sulforaphane	16.27
	Total	30.47
Black radish	Allyl ITC	4.62
	Isobutyl ITC	10.28
	benzyl ITC	7.52
	Raphasatin	3.24
	Phenethyl ITC	3.54
	Total	29.2

Table 5. Effect of cruciferous plant (turnip, arugula, broccoli, and black radish) extracts at a concentration of 20% on germination indicators of field dodder seeds

Treatments	G%	L/cm	SG	MGT	MDG	PV	GV
Ctrl	49.6^{c}	5.16^{d}	2.68^{c}	9.15^{c}	1.50^{c}	0.30^{b}	0.49^{b}
Turnip 20%	2.1^{a}	0.40^{a}	0.09^{a}	0.45^{a}	0.06^{a}	0.06^{a}	0.01^{a}
Arugula 20%	9.6^{b}	0.76^{b}	0.45^{b}	1.95^{b}	0.29^{b}	0.11^{a}	0.04^{a}
Broccoli 20%	10.4^{b}	1.01^{bc}	0.57^{b}	1.84^{b}	0.31^{b}	0.10^{a}	0.04^{a}
Black radish 20%	12.5^{b}	1.16^{c}	0.62^{b}	2.39^{b}	0.38^{b}	0.10^{a}	0.04^{a}
LSD 0.01	5.211	0.334	0.255	1.269	0.152	0.068	0.068

Values followed by the same letter(s) in the same column are not significantly different from each other at 0.01 level of probability, (G: field dodder seeds germination, L: the length of field dodder shoots, SG: speed of germination, MGT: mean germination time, MDG: mean daily germination, PV: peak value, GV: germination value).

4. Discussion

All extracts of cruciferous plant at a concentration of 2% illustrated a slight effect in the germination of field dodder seeds compared to control plots. As well as all extracts at the same concentration did not affect the shoot length of field dodder nor the speed of seed germination. The other indicators such as MGT, MDG, PV, and GV all decreased under the effect of turnip and arugula extracts at a concentration of 2%, whereas these indicators were not affected by broccoli and black radish extracts at the same concentration.

With increasing the concentration of the extracts to 6%, all the studied germination indicators decreased compared to the control plots, while the peak value was not affected by black radish extract.

At a 10% concentration of cruciferous plant extracts, field dodder seed germination decreased significantly compared to the control plots. Turnip extract had the lowest germination rate at 9.6%, while black radish had the highest at 14.5%. These extracts also affected shoot length, with turnip and arugula having a stronger impact than broccoli and black radish. Other germination indicators (SG, MGT, MDG, PV, and GV) also decreased, with black radish showing a lesser effect on MGT and MDG.

At a 20% concentration of turnip extract, field dodder seed germination rate dropped to 2.1%, while arugula, broccoli, and black radish extracts at the same concentration resulted in germination rates ranging from 9.6% to 12.5%. These extracts also significantly affected field dodder shoot length, ranging from 0.40 cm (in turnip) to 1.16 cm (in black radish). Other germination indicators (SG, MGT, MDG, PV, and GV) were also significantly reduced by the 20% cruciferous plant extract treatments compared to control plots. It should be noted that high concentrations of cruciferous plant extracts may have a highly negative osmotic potential that may affect seed germination. Using a control with PEG solution may give the same osmotic potential effect to avoid any other influences in seed germination experiments.

Cruciferous plant extracts at various concentrations reduced field dodder seed germination and other indicators due to allelochemicals produced from GLS hydrolysis. Previous studies, like Brown and Morra (1996), have reported complete inhibition in weed seed germination by brassica species' hydrolysis products. Uremis et al. (2009) confirmed the allelopathic potential of six brassica species in controlling Johnson grass in both lab and field conditions.

Turnip had the highest ITC compound content at 56.6% among the studied cruciferous plants, with six compounds identified: allyl ITC, 3-butenyl ITC, cyclopentyl ITC, heptyl ITC, phenethyl ITC, and octyl ITC. Previous studies also found multiple ITC compounds in turnip, aligning with our results. These compounds vary across plant parts, including roots (n-butyl ITC, 3-butenyl ITC), leaves and flowers (benzyl ITC, allyl ITC, 4-pentenyl ITC), and the whole plant (2-phenylethyl ITC), as reported by Petersen et al. (2001) and Paul et al. (2019). Vieites-Outes et al. (2016) identified eight ITC compounds in turnip, reinforcing our findings.

Arugula contained a total of 36.23% ITC compounds, including benzyl ITC, phenethyl ITC, sulforaphane, and 1-naphthyl ITC. Previous studies confirmed the presence of phenethyl ITC in arugula resulting from gluconasturtiin hydrolysis (Ioannides et al., 2010). Other studies also found four ITC compounds in arugula, including phenethyl ITC, allyl ITC, iberin, and sulforaphane (Villatoro-Pulido et al., 2013; Rodrigues et al., 2016), partially aligning with our findings.

Broccoli contained 30.47% ITC compounds, including allyl ITC, raphasatin, iberin, and sulforaphane. These results partially align with previous study reporting three ITC compounds (allyl ITC, phenethyl ITC, iberin) in broccoli (Wang et al., 2010). Other previous studies found various ITC compounds in broccoli, such as sulforaphane, iberin, erucin, allyl ITC, and phenethyl ITC (Liang et al., 2006; Lv et al., 2021) which partially support our findings.

Black radish had 29.2% ITC compounds, including allyl ITC, isobutyl ITC, benzyl ITC, raphasatin, and phenethyl ITC. These results align with Elsekran et al. (2023) who found five ITC compounds in black radish (tert-butyl ITC, 2-propenyl ITC, benzyl ITC, 4-methylthio-3-butenyl ITC, 2-phenylethyl ITC) totaling 40.39%. Our study partly concurs with Uremis et al. (2009), who reported that black radish contains high levels of benzyl ITC and allyl ITC. Additionally, Castro-Torres et al. (2013) reported two ITC compounds in black radish, raphasatin and sulforaphane, partially matching our findings.

5. Conclusion

Cruciferous plant extracts, at varying concentrations, significantly decreased field dodder seed germination and other germination indicators, such as shoot length, germination speed, mean germination time, mean daily germination, peak value, and germination value. The reduction correlated with higher extract concentrations and ranked from highest to lowest efficiency as follows, turnip, arugula, broccoli, and black radish. The allelopathic effects of cruciferous plants can be used to control field dodder as an environment friendly alternative control method instead of using herbicides. It is recommended to adopt cruciferous crops in agricultural rotations or to cultivate these cruciferous plants in fields infested with field dodder, then incorporate these plants into the soil at certain stages before cultivating the main crop to benefit from their allelopathic effects in reducing the soil's stock of field dodder seeds.

Author's contributions

All authors read and agreed to the published version of the manuscript. KA: contributed to implementing all field and laboratory works, data collection, data analysis, and writing the manuscript. TU: supervised the experiment and reviewed the manuscript

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