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Research Article

Assessing allelopathic potential: *Boreava orientalis* impact on *Triticum aestivum*

Taiebeh Adeli[1], Iraj Tahmasebi[1], Sirwan Babaei[1, 2*] and Amir Sadeghpour[2]

1. Department of Plant Production and Genetics, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran.
2. Crop, Soil, and Environmental Management Program, School of Agricultural Sciences, Southern Illinois University, Carbondale, IL, USA 62901.

Abstract: Waxy-leaved mustard (*Boreava orientalis* Jaub. and Spach.) is an invasive species recently reported in Kurdistan province in western Iran with allelopathic properties. To evaluate the effect of the extract of different parts of waxy-leaved mustard on wheat germination, an experiment was conducted as a factorial based on a completely randomized design in the Faculty of Agriculture, University of Kurdistan, Iran. The allelopathic potential of waxy-leaved mustard's root, leaf, stem, and flower were evaluated, and secondary metabolite compounds were identified. Effect of Alcohol extracts at concentrations of 0, 1, 2, 3, and 4% of roots, leaves, stems, and flowers of waxy-leaved mustard were evaluated on wheat germination Indicators. Increasing extract concentration significantly increased the inhibition of seed germination and caused a decrease in germination rate, reduction of stem length, and reduction of seed vigor index. Alcoholic extracts of waxy-leaved mustard flowers had significantly more effect compared to the alcoholic extracts of roots, even at the lowest concentration (1%). The results of this study show that waxy-leaved mustard has a strong allelopathic potential, which emphasizes the importance of reducing its allelopathic effects and developing effective management strategies for mitigating invasion risk and thereby protecting crops like wheat.

Keywords: Allelopathy, alcoholic extracts, Brassicaceae, waxy-leaved mustard

Introduction

The cultivated crops suffer more damage than other plants in a region from the allelopathic effects of the weed plants. For this reason, these alien plants succeed and spread in competition against the cultured crops (Zhang *et al*., 2021). Secondary compounds produced by plants are often considered an alternative approach to weed suppression (Yuan *et al*., 2021). Allelopathy is an interference mechanism due to which living or dead plants release chemicals that inhibit or stimulate the growth of surrounding plants (Das *et al*., 2012).

Waxy-leaved mustard *Boreava orientalis* Jaub. and Spach., commonly known as yellow weed, is a species of the Brassicaceae family predominantly found in wheat fields in central Turkey. However, this weed is also distributed worldwide, including in countries such as

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*Corresponding author: s.babaei@uok.ac.ir
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France, Belgium (CABI, 2022), and Iran. In "The Flora of Iran" this species is introduced as the synonym of *Isatis quadrualata* (Moazzeni *et al*.,2010). Waxy-leaved mustard is an invasive species recently reported in Kurdistan province in western Iran, close to Turkey (Babaie *et al*., 2020). Fatty acids, Glucosinolates, and conjugated Oligosaccharides with Hydroxycinnamol groups have been found in Waxy-leaved mustard seeds. Isolation and identification of Hydroxybenzoic acid and Glucoside, 3,2-Dihydroxybenzoic acid, which is an antioxidant, have been done inform seeds of this plant (Sakushima *et al*., 1995; Tank *et al*., 1994).

Seed germination is a critical stage in the life cycle of plants, and its successful establishment in an agricultural ecosystem is essential (Windauer *et al*., 2007; Babaei *et al*., 2022). Allelopathic substances inhibit germination in surrounding plants, and many studies have shown this phenomenon (Smallegange *et al*., 2007; Sen *et al*., 2022; Oliwa *et al*., 2017). Previous studies have suggested that the seeds of waxy-leaved mustard have allelopathic properties (Sakushima *et al*., 1995; Tank *et al*., 1994).

Therefore, this study aimed to investigate (i) whether other parts of Waxy leaved mustard have secondary metabolites that can be allelopathic (ii) to what extent the secondary metabolites in plant part extracts can affect weeds and wheat germination percentage, germination rate, seedling length, and seed vigor index because this weed is often present in wheat fields and has spread greatly, and wheat is an important crop in western parts of Iran, so investigating the effect of secondary metabolites in waxy mustard on wheat germination characteristics is of important.

Materials and Methods

The experiments were conducted in two sections within the Department of Plant Production and Genetics at the University of Kurdistan, Iran, under controlled laboratory conditions from 2019 to 2020. The initial phase involved extracting various components of waxy-leaved mustard to assess their impact on germination indicators in wheat. The subsequent phase focused on material analysis through Gas Chromatography Mass Spectrometry (GC-MS).

Plant material

Waxy-leaved mustard plants were collected from wheat fields in Kurdistan (18.35 °N, 18.47 °E). The plants were harvested in July 2018. Ten specimens of waxy-leaved mustard were gathered, encompassing all plant organs, which were subsequently divided into flowers, roots, stems, and leaves. Sampling was conducted across a 1 m^2 area in five distinct field locations, with ten plants selected from each location. Mature waxy-leaved mustard has 35% (flower weight /the whole plant) flowers, 20% leaves (leaves weight /the whole plant), 25% stems (stems weight /the whole plan), and about 20% roots (roots weight /the whole plan). To prepare the alcoholic extract, plant parts must be dried, so the dry weight of these plant parts was used. To identify phytochemical substances by GC Mass, it is necessary to prepare an alcoholic extract.

Alcoholic extracts

Different plant parts were separated. The parts of the plant were washed and air-dried. After separating the leaves and buds from the stem, the plant parts were dried for 48 hours at room temperature and then ground into a fine powder using an electric grinder. The powder was sieved through a 0.5-micron sieve, and the resulting material was used to prepare the alcoholic extract. The powdered plant material was mixed with 96% ethanol at 1:10 (w/v) and placed in an Erlenmeyer flask to extract the compounds. The flask was covered with aluminum foil to prevent light penetration and left on a hotplate at room temperature for 72 hours until the extract was dissolved entirely. The alcoholic extract underwent filtration using Whatman filter paper and a Buchner funnel, and the liquid obtained from the rotary evaporator was heated to 50 °C to facilitate the separation of alcohol from the plant extracts. The mixture was distilled to concentrate the plant extracts as much as possible. The concentrated extract was maintained at 4 °C until used (Narwal *et al*., 2009). In total, 64 extracts (plus 16 controlled treatments with 0% concentration) at different concentrations were prepared. The weight/volume was used to prepare the extracts. The prepared extracts were stored in a refrigerator at 4 °C until analyzed by a GCMass device (in Erlenmeyer flasks covered with aluminum foil).

Gas chromatography

Extracts obtained from different plant parts were analyzed by Gas chromatography with mass spectrometry (GCMass) (AGILENT, USA) to identify and analyze phytochemicals. The presence of secondary metabolites has cast a shadow on innovative, unique methods from capillary to multidimensional GC. Capillary GC has given enigmatic conditions by giving narrow peaks of numerous compounds, though capillary GC was positive for identifying organic compounds. However, novel techniques should be innovated to observe the complexities of structural analysis. Multidimensional GC (MDGC) has been a successful technique for isolating aromatic compounds. Capillary GC has rapidly replaced packed GC for aromatic compound description. GC coupled with MS and flame ionization detection (FID) have been effectively smeared to characterize aromatic compounds (Marriott, 2001). Then, the extracts obtained from different parts of the plant were used to check and identify the substances by Gas chromatography. This analysis identified the biochemical substances in different plant parts, including flowers, stems, leaves, and roots. All the aromatic compounds in different plant parts were measured using the same method in the sequence explained by Waseem and Hin (2015).

Determination of several analyses from different samples (flowers, leaves, stems, and roots):

This extraction method is faster than the traditional solvent-based approaches. One line-coupling of the SFE-GC system contains the following steps.

a. Extraction of the analyses by S-CO_2.

b. Trapping of extracting analyses into an intermediate trap (SPE).

c. Transferring of trapped analyses into a GC column or injector.

d. Separation of the target analyses by GC.

Data analysis

The experiment was conducted as a factorial design based on a completely random design with four replications. Factors contained different plant parts such as flowers, leaves, stem, and root, and different extract concentrations. After making the extracts, four mL of the extracts of different parts of waxy-leaved mustard were added to each petri dish (10 cm diameter glass Petri dishes containing 25 wheat seeds). The samples were placed in the germinator and kept at a controlled temperature (25 °C degrees) and light conditions (16 light/8 dark). Seeds were counted daily, which continued for one week. Then, the data that was obtained was analyzed (Capuani, 2012; Oliwa, *et al*., 2017). The studied traits included germination percentage (GP), germination rate (GR), Seedling vigor index (SVI), and seedling length (SL), which are obtained with the following formulas (Lu, 2011):

Eq 1: $GP\ (\%) = \frac{\text{No of germinated or emerged seedling}}{\text{Days of first count}} + \cdots + \frac{\text{No of germinated or emerged seedling}}{\text{Days of final count}}$

The germination rate (GR) was calculated as follows:

Eq 2: $GR\ (\%) = n / N \times 100$

n: The number of germinated seeds

N: Total seeds

Eq 3: $SVI = (SL \times GP) / 100$

Eq 4: $\text{seedling length (SL)} = \text{length of Radicle} + \text{length of Plumule}$

The data were organized in Excel, and normality tests for GP, GR, SVI, and SL indices were conducted using the Kolmogorov-Smirnov test ($P > 0.05$). Subsequent analysis was performed using SAS (Version 8) (Statistical Analysis System). Data analysis and analysis of variance were performed using SAS by ANOVA, which was done using a factorial design based on a completely random design and comparison of means at $P \leq 0.05$ probability level. All statistical analyses were performed using SAS. Three-way ANOVA was used to compare differences among treatments. When the ANOVA showed significant differences, we used the LSD test for pairwise comparisons. The level of significance was fixed at $P < 0.05$. By checking the obtained R^2, the appropriate graph was fitted. The fitted models were linear and Exponential decay, two-parameter models with Equations as follows (Zhang *et al*., 2021 and Qiao *et al*., 2023).

Eq 5: $f = y0 + a \times x$

y_0 = is the time required for 50% germination, a= The slope of the line

Eq 6: $f = a \times \exp(-b \times x)$

A = The curve's maximum value, b= The slope.

Results

Germination percentage

All extracts of waxy-leaved mustard significantly ($P \leq 0.05$) decreased seed germination (Figure 1). Comparing the slope of the linear regression lines showed that the slope related to the flower had a more decreasing trend than the other parts. The slope of the flower was determined to be -14.97. By increasing flower extract concentration, the GP of wheat decreased. Subsequently, the slope of the leaves exhibited a similar downward trend, with a value of -13.6. On the other hand, the slopes of the stem and root were found to be almost equal. The highest GP was 73.5%, which was related to the concentration of extract from the plant's root; so, the flower and leaves of waxy-leaved mustard had more inhibitory effects than the roots and stem. Most of the secondary metabolites are found in the flower of the waxy-leaved mustard. Identifying the plant parts and effect of concentrations of extracts informs growers about management tactics for reducing the allelopathic effects of the waxy-leaved mustard while offering opportunities for harnessing the allelopathic potential of this plant for effective bioherbicide production to control weeds. Probably due to presence of isothiocyanate only in flowers (Table 1), this extract showed a more significant effect than other extracts in reducing wheat germination indices. Leaves and flowers are probably the primary sources of allelopathic substances (Isothiocyanate). The roots have smaller amounts of these compounds, and 1-Butano, 4-Isothiocyanate is 2.204%, which is one of the reasons thet germination affected by root extract was higher than the germination affected by flower extract (Table 1).

Germination rate (GR)

an alcoholic extract obtained from waxy-leaved mustard caused a decrease in the rate of wheat germination, as stated in (Figure 2). A linear model best described the relationship between the concentration of the extracts and the GR. Figure (2) presents the graph depicting the effect of waxy-leaved mustard extracts on the GR of wheat. The slope related to flowers was -6.88, showing a decreasing trend higher than the other parts. The slope of the line related to the leaves was almost like the flower(-6.29). The stems also showed a greater decreasing slope than the roots, which was -4.51. The root had the lowest slope value, least affecting the GR. The highest GR was 32.82 compared to the control, and the lowest was 2.78, obtained at a concentration of 4% flower extract. Compared to the other parts, flowers caused a decrease in the germination rate, and the lowest effect was observed by the root, which means it had a minor effect on the germination rate.

Seedling vigor index (SVI)

The seedling vigor index (SVI) decreased as the plant extract concentration increased (Figure 3). Comparing the slopes of the lines on the SVI chart indicated that the line associated with the flower had the highest decline, with a slope value of 0.99. Next to the flower, the leaves had the most significant impact on the SVI, which was 0.81. Roots and leaves had an almost equal slope of the line.

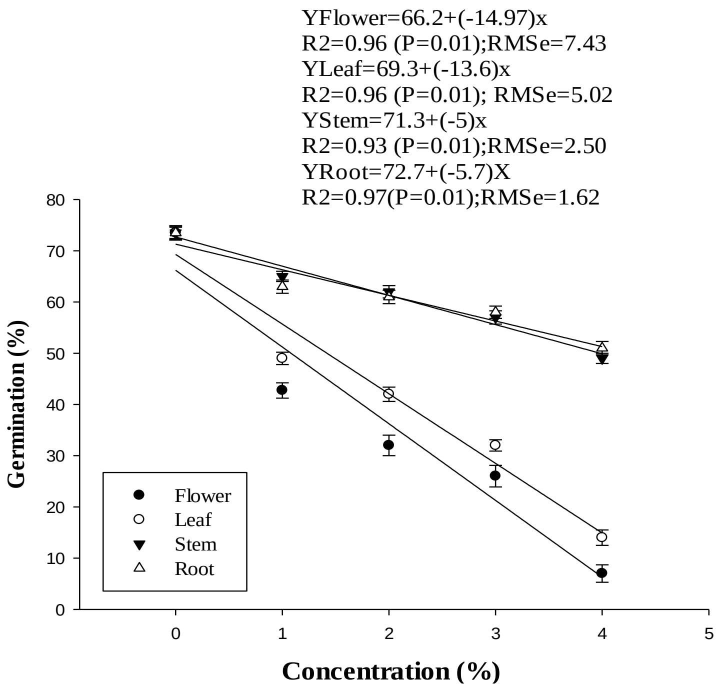

Figure 1 The effect of extracts of *Waxy leaved mustard* on germination percentage of wheat seed.

Table 1 Quantification of biochemical substances detected in different parts of waxy leaved mustard using GCMass.

Materials	Amount of material in different parts of the plant (%)			
	Flower	Leaf	Stem	Root
Decanoic acid	29.559	0	0	0
Undecenoic acid	7.373	0	0	0
Dodecanoic acid	5.846	0	0	0
Amino1-methyl	4.114	0	0	0
3, 9-dimethyl	3.656	0	0	0
1, 3-Dioxalan	3.284	0	0	0
Hexahydropyrrolysin	2.872	0	0	0
Tetradecan	2.675	17.507	6.838	14.116
Hydroxylamin	2.526	23.998	0	7.168
3, 5 Dimethyl octan	2.512	0	0	0
Benzen ethan amin 2,3-difluoro beta	2.431	0	0	0
1-butano,4-isothiocyanate	2.204	0	0	0
Tetramethyl hexa decan	0	52.76	0	0
Benzen propan nitril	0	0	23.934	0
Naphthalen carboxylic acid	0	0	11.645	0
Dodecan	0	0	11.053	0
Bicyclopentan	0	0	7.605	0
Benzene dialdehyd	0	0	23.934	0
Cyclopentasiloxan	0	0	11.645	0
Benzoic acid,2-(methylamino), propyl ester	0	0	11.053	0
α- arginin	0	0	7.605	0
Cyclohexa	0	0	6.229	0
Cyclohexanol	0	0	6.229	0
Benzen propan nitril	0	0	0	22.877
Naphthalen carboxylic acid Bicyclohepentane	0	0	0	15.678

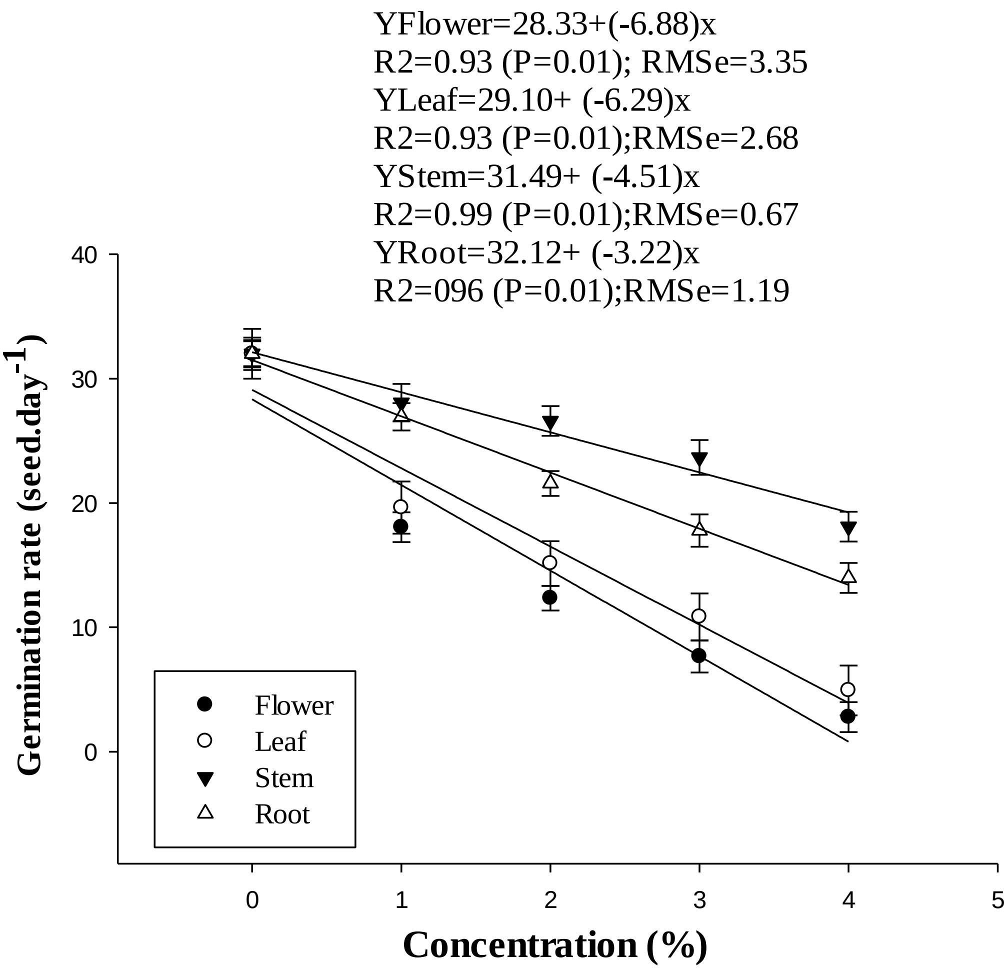

Figure 2 The effect of extracts of *Waxy leaved mustard* on the germination rate of wheat seed.

The highest value of the SVI was 16.68, and the lowest value was related to the treatment of 4% flower extract, which is 0.075. Fig. (3) shows the slopes related to the equation of the effect of allelopathic substances in different parts of waxy-leaved mustard; all the parts were fitted to the logistic model because the other models were not significant for them. The highest slope value (1.731) is related to the flower, significantly reducing SVI. The slope of the line in the flower decreased faster, so it can be concluded that it contained more allelopathic substances. The lowest value of the slope is (0.7901) in the stem, which is less effective on SVI than on other parts. Hydroxybenzoic acid derivatives are present in waxy-leaved mustard, and by reducing the SL and GP, the value of the SVI is also decreased.

Seedling Length (SL)

The SL was reduced and affected by all the concentrations of the alcoholic extracts (Figure 4). The diagram related to SL was fitted to the Exponential decay. Flowers and leaves extracts decreased the SL with higher intensity, and the line slope for these parts showed a more significant decreasing effect than the stem and root. The slope of the line was 0.55 in flowers and 0.48 in leaves. The line slope was 0.07 in the stem and 0.08 in the root, which had less effect on the SL. Stem length was sensitive to all extracts and was significantly reduced by waxy-leaved mustard at all concentrations ($P < 0.05$).

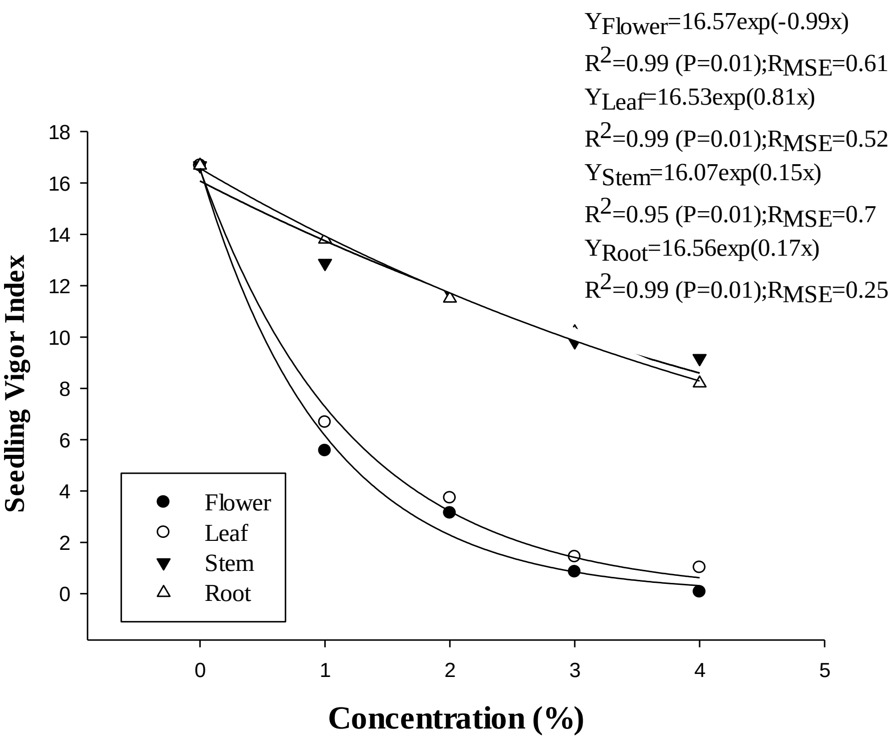

Figure 3 The effect of extracts of *Waxy leaved mustard* on Seedling vigor index (SVI) of wheat seed.

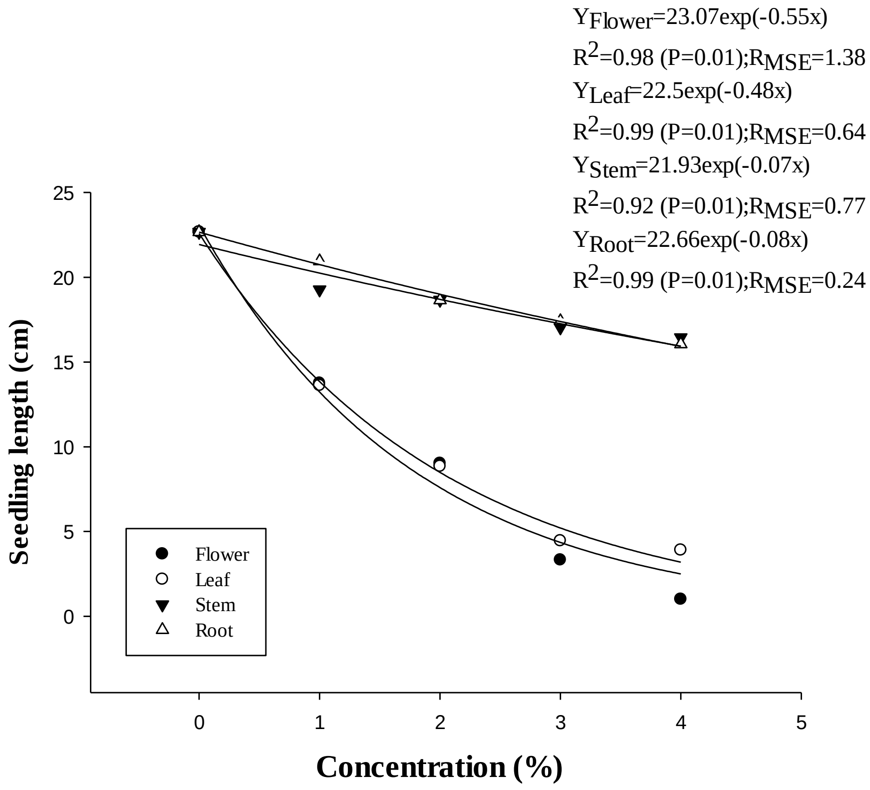

Figure 4 The effect of extracts of *Waxy leaved mustard* on seedling length of wheat seed.

Discussion

Secondary metabolites in plant parts

Isothiocyanate was identified and was present only in the flower (Table 1). The other Secondary metabolites that were found only in the flowers included isothiocyanate (2.20%), Decanoic acid (29.5%), and Undecanoic acid (7.37%).

The effect of these compounds has been shown in species of the Brassicaceae family, such as *Sinapis arvensis* (Brijacak *et al*.,2020), *Cardaria draba* (Qasem, 2016), and *Brassica nigra* (Turk and Tawaha, 2002; Al-Sherif and Hegazy, 2013). The Brassicaceae family has allelopathic effects that inhibit germination in other plants. In *Pieris brassica*, the flower contains more isothiocyanate compounds than other parts of the plant (Smallegange *et al*., 2007). The allelopathic effect of wild mustard was investigated, the compounds were detected, and the highest rate of allelopathic extract was in the parts of flowers and leaves (Haddadchi and Khorasani, 2006).

Some allelopathic compounds, such as isothiocyanate or their relatives, are also present in waxy-leaved mustard (Table 1). The mature waxy-leaved mustard is nearly 50 cm tall, and most of the plant volume is related to the flower. The ratio of flowers to the whole plant was about 35%, leaves 20%, stems about 25%, and roots about 20% of the plant. In comparing the different parts of waxy-leaved mustard with wild mustard, which are very similar, the volume of the flowers in wild mustard is smaller than that of waxy-leaved mustard. However, the leaves of waxy-leaved mustard are smaller than those of wild mustard, but the roots in both plants are almost the same size. Therefore, the size of the flowers is larger in the waxy-leaved mustard. Naturally occurring allelopathic substances, with isothiocyanate (2.20%) being the most prominent, were found exclusively in this particular part of the plant, demonstrating higher concentrations.

Sulfured and nitrogenous compounds, such as isothiocyanate derivatives, are recognized as secondary metabolites in plants (Waseem and Hin, 2015). Consequently, when these flowers are introduced into the soil, they can adversely affect wheat yield in agricultural settings. The flowers, possessing a higher concentration of various substances essential for effective allelopathic control and a greater biomass percentage, exert a more pronounced influence on growth indicators in wheat.

In this study, it was shown that waxy-leaved mustard has allelopathic properties and affects the germination of wheat seeds. This could depend on the types of relationships with allelochemicals among different botanical families (Lorenzo *et al*., 2010; Hussain and Reigosa, 2011) or the type of allelochemicals or compounds present in the extracts (Latif *et al*., 2017). This study showed that leaf and flower extracts of waxy-leaved mustard have more allelopathic effects on wheat seed and seedling germination than root and stem extracts, which was also reported by Soudaizadeh *et al*. (2009). While secondary compounds with allelopathic potential are produced in various plant tissues, including seeds, stems, roots, etc., leaves seem to be the main source of chemicals involved in phytotoxicity (Suzuki *et al*., 2017; Ali *et al*., 2019; Hossein *et al*., 2020). Allelochemicals appear to alter many physiological processes (Mushtaq *et al*., 2020). Allelopathic interactions generally result in delayed or limited seed germination, which usually depends on the concentration of the active ingredients present (Oliwa *et al*., 2017). Species of the Brassicaceae family can inhibit the germination and growth of other plants. Biological activity is frequently associated not directly with glucosinolates but rather with other compounds resulting from enzymatic hydrolysis, including organic cyanides (CN), oxazolidinethiones (OZT), isothiocyanates (ITC), and thiocyanate ions (SCN) (Brijacak *et al*., 2020). Isothiocyanates are one of the secondary metabolites produced by Brassicaceae species. The aqueous extract of *Brassica rapa* reduced the germination rate and seedling growth of winter wheat and corn *Zea mays* L. between 26.5 and 79.5% (Suzanne *et al*., 2000). The extracts of *Echinochloa crus-galli* with mixed or single aqueous extracts of Brassicaceae species reduced germination (Smallegange *et al*., 2007; Suzanne *et al*., 2000). The inhibitory effect of the aqueous extract of the leaves of several Brassicaceae species on the germination of soybean *Glycine max*, wheat, and corn was proven in pot experiments (Zukalová and Va, 2002). These compounds produced in the root of *Cardaria draba* are a bioactive inhibitor for the germination of other species. These compounds affect the germination of cereal seeds. Compounds such as isothiocyanate created by the hydrolysis of glucosinolates are produced under the influence of myrosinase enzyme. They are most crucial in reducing the germination rate (Sen *et al*., 2022). These compounds primarily target enzymes within the glycolysis pathway, and their presence at low respiratory concentrations hinders or inhibits the germination process. Allelopathic substances at low concentrations may have positive or negative effects on target plants, but they are always inhibitory at high concentrations. In this experiment, high concentration was more inhibitory and strongly reduced Germination rate, and GR was more affected by allelochemicals than germination percentage (Oliwa *et al*., 2017). *Brassica napus* reduces soybean seedlings' germination, height, and dry weight (Qassem, 2016).

The increase in extract concentration adversely impacted seedling growth, leading to a decrease in its overall magnitude. This reduction was probably due to the inhibitory effect of *Boreava* allelochemicals on the absorption and translocation of minerals (Turk and Tawaha, 2002). Other compounds cause damage by interfering with vital physiological processes, such as preventing cell division and the activity of certain enzymes (Singh *et al*., 2008). Impaired absorption of nutrients, plant hormones, respiration, and RNA and DNA structure changes can reduce stem and root length. In general, root length can be reduced, indicating that the allelopathic agents interfere with the action of gibberellin and IAA, thereby inhibiting cell elongation (Zhang *et al*., 2021; Waseem and Hin, 2015). Wheat SL was affected by all extracts (flower, stem, leaf, and root) at all concentrations of black mustard. Extracts from different parts of the allelopathic plant showed a significant difference in phytotoxicity to wheat seedlings (Turk and Tavaha, 2002). Research on *Brassica nigra* L., commonly known as black mustard and found in annual grassland fields along the southern coast of California, revealed its ability to germinate by releasing inhibitors during decomposition. These inhibitors hinder the growth of other annual plants, such as *Bromus* (Brijacak *et al*., 2020). Black mustard extract reduced SL in wheat and Egyptian clover (*Trifoiium alexandrium* L.) even at low concentrations, which was due to the presence of isothiocyanate, syringic, and ferulic acids, which were the dominant substances present in HPLC (Gassim, 2016). The presence of isothiocyanate was similarly confirmed in this experiment. Like other plants of the Brassicaceae family, wild mustard has glycosidic compounds that have an inhibitory effect on the growth of many plants. It has been reported that wild mustard flower and stem extracts decreased the growth of stems, roots, leaves, and fresh weight of rapeseed (Zhang and Gosta, 2010). We concluded that waxy-leaved mustard is potentially allelopathic as it inhibits wheat seed germination; treatment with different concentrations of waxy-leaved mustard extract has an inhibitory effect, which is evidence for allelopathy. The flowers have the most allelopathic substances for effective germination control and make up 35% of its biomass, more than stems, roots, and leaves. Flowers were found to have the greatest effect on wheat growth indices among all plant parts, probably due to the presence of isothiocyanate (2.20%), benzene ethaneamine, 2,3-difluorobeta (2.431%), decanoic acid (2.431 %). 29.5%, and undecanoic acid (7.37%). These findings show that if the waxy mustard flower enters the soil, it can significantly reduce wheat germination, growth, and yield.

Conflict of interest statement

The authors declare that they have no known conflict of interest that could influence the work reported in this paper.

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ارزیابی پتانسیل آللوپاتیک *Boreava orientalis* Jaub. and Spach بر روی *Triticum aestivum* L.

طیبه عادلی[1]، ایرج طهماسبی[1]، سیروان بابائی[1,2]* و امیر صادق‌پور[2]

۱-گروه مهندسی تولید و ژنتیک گیاهی، دانشکده کشاورزی، دانشگاه کردستان، سنندج، ایران.
۲- گروه مدیریت خاک، محصولات کشاورزی و محیطزیست، دانشکده علوم کشاورزی، دانشگاه ایلینوی جنوبی، ایالات متحده آمریکا.
پست الکترونیکي نویسنده مسئول مکاتبه: s.babaei@uok.ac.ir
دریافت: ۲ مرداد ۱۴۰۲؛ پذیرش: ۲۳ آبان ۱۴۰۲

چکیده: خردل برگ مومی گونه‌ای مهاجم است که اخیراً در استان کردستان گزارش شده است که دارای خواص دگرآسیبی است. به‌منظور ارزیابی اثر عصاره قسمت‌های مختلف خردل برگ مومی بر روی جوانه‌زنی گندم آزمایشی در قالب فاکتوریل بر پایه طرح کاملاً تصادفی در دانشکده کشاورزی دانشگاه کردستان، ایران انجام شد. پتانسیل آللوپاتیک ریشه، برگ، ساقه و گل خردل برگ مومی بررسی و ترکیبات متابولیت‌های ثانویه شناسایی شد. عصاره‌های الکلی در غلظت‌های ۰، ۱، ۲، ۳ و ۴ درصد از ریشه، برگ، ساقه و گل خردل برگ مومی بر روی شاخص‌های جوانه‌زنی گندم استفاده شد. افزایش غلظت عصاره به‌طور معنی‌داری باعث مهار جوانه‌زنی بذر و کاهش سرعت جوانه‌زنی، کاهش طول ساقه و کاهش شاخص بنیه بذر گردید. عصاره الکلی گل خردل برگ مومی به‌طور قابل‌توجهی در مقایسه با عصاره الکلی ریشه حتی در کم‌ترین غلظت (۱%) اثر بیشتری نشان داد. نتایج این مطالعه نشان می‌دهد که گل خردل برگ مومی دارای پتانسیل آللوپاتیک قوی است که بر اهمیت کاهش اثرات آللوپاتیک آن و توسعه استراتژی‌های مدیریتی مؤثر برای کاهش خطر تهاجم و حفاظت از محصولات زراعی مثل گندم تأکید می‌کند.

واژگان کلیدی: آللوپاتی، عصاره‌های الکلی، خانواده شب‌بو، خردل برگ مومی