



## Allelopathic effects of *Palustriella falcata* (Bryophyta) extracts on wild mustard plants

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### Abstract

In order to protect themselves from various stress factors in certain habitats, bryophytes produce secondary metabolites such as phenolics. This study aimed to determine the effects of *Palustriella falcata* (Brid.) Hedenäs on growth parameters and levels of photosynthetic pigments, total protein, proline and total phenolics of *Sinapis arvensis* L. (wild mustard) to be used for biological control. There are non-significant differences in growth parameters compared to controls. The levels of photosynthetic pigments generally increased in the *P. falcata* distilled water treatments; decreases occurred in the other solvent treatments. The biggest decreases were found for the treatments with ethyl acetate and 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate. The total protein amount and peroxidase enzyme activity are opposite to each other. Increases in the amount of proline and total phenolics were determined in all treatment groups. In the *P. falcata* treated groups, the biggest increases were seen with the 25mg.mL<sup>-1</sup> ethyl acetate treatment (44.25% increase in total protein amount and 49.82% in proline amount), and with the 50mg.mL<sup>-1</sup> ethyl acetate treatment (1050.76% increase in total phenolic amount). It is thought that the changes observed are due to the allelopathic effect of the *P. falcata*.

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## Introduction

It is important to develop less environmentally harmful methods for weed control, due to the harmful effects of commercial herbicide use. Many plants have been shown to have inhibitory effects on the growth of adjacent plants (Einhellig, 2004; Uddin and Robinson, 2017). The biochemicals involved, known as allelochemicals, such as phenolic compounds, terpenoids, triterpenoids, alcohol derivatives and flavonoids, can directly or indirectly influence the growth and development of neighbouring organisms (Srivastava, 2015).

There are approximately 15,000 species of bryophytes worldwide (Gradstein *et al.*, 2001; Alam, 2015), making them the most diverse group of plants, after flowering plants. Mosses are comprised of various classes, totalling 10,000 or more species (Koua *et al.*, 2015). Bryophytes are found in all ecosystems except in the marine ecosystem, and are regularly exposed to different abiotic and biotic stress factors. Most of the secondary metabolites produced by bryophytes, especially terpenoids and phenolic compounds, are synthesized as defense systems (Basile *et al.*, 2003). Bryophytes play a major role on the germination of vascular plant seeds falling on (Glime, 2007), and some higher plants do not grow well in the vicinity of bryophytes (Kato-Noguchi *et al.*, 2010). Asakawa (2007) postulated that higher plants sometimes do not grow in places inhabited by specific bryophytes because of allelopathic compounds, and Wang *et al.* (2016) explained that allelochemicals of bryophytes can impart growth regulatory activities onto other plants. Inhibitory effects of bryophyte extracts on the growth of rice, wheat, lettuce and radish are also known (Basile *et al.*, 2003). The degree of inhibition was dependant on the concentration of the allelopathic compounds (Sharma *et al.*, 2009; Wang *et al.*, 2013).

Yield and quality is reduced due to negative correlation between crops and weeds in agricultural areas. *Sinapis arvensis* L. reduce the yield and quality in the cereal production areas in our region and country, and adverse effects on economy. In recent years, biological control of these weeds has gained great importance.

There are studies on the use of vascular plants in weed control. The aqueous extracts of chamomile, dandelion and nettle inhibited the seed germination and seedling growth of *S. arvensis* (Khatami *et al.*, 2017). Bryophytes are plants that contain significant amounts of secondary metabolites and affect the development of many vascular plants. This effect of bryophytes was not studied much at the physiological and biochemical level. The aim of the study was to investigate allelopathic effects of *P. falcata* extracts on wild mustard. For this purpose, photosynthetic pigment substances, total protein, proline and total phenolics of wild mustard were measured and compared.

## Materials and methods

### Plant materials

*P. falcata* were collected from rocks in Kayseri, Yahyalı, Kapuzbaşı Team Falls (Elif Falls) in 2015. The plants were identified using the relevant literatures (Smith, 2004; Cortini Pedrotti, 2006). They were cleaned of rocks, soil and weeds, washed with distilled water, and dried at room temperature for several days. Moss specimens (0.5g and 1g, powdered in liquid nitrogen) were extracted with 20mL of solvent [distilled water, ethanol (Sigma-Aldrich) and ethyl acetate (Sigma-Aldrich)]. The samples were incubated in solvent for 1h, filtered through Whatman no: 2, then the solution was sprayed onto the wild mustard plants until the leaves are completely soaked.

Wild mustard (*Sinapis arvensis*) seeds were collected from Adana-Imamoglu (Turkey) in 2015. The plants were identified using the flora and overhaul studies (Davis, 1965).

### Growing conditions, experimental design and sampling

The study was carried out in plant growth chamber [constant humidity (50±5%), 16: 8 photoperiod and 23±2°C temperature] in the biotechnology department of Nigde Omer Halisdemir University. The experimental design was randomly with three replicates. The seeds were watered every other day, and allowed to germinate. Seedlings at 20-days-old were treated with 0,25 and 50mg. mL<sup>-1</sup>

concentrations of the bryophyte extracts applied as foliar at 2-day intervals. The plants were harvested for growth measurement and analysis when they were 30-days-old.

#### *Photosynthetic pigment amount analysis*

Chlorophyll and carotenoid were extracted from 0.5g fresh leaves by homogenizing the leaves in 5mL 80% (v/v) acetone in the dark, followed by filtration. Absorbance values were measured at 663 and 645nm wavelengths for chlorophyll a and b, and at 450nm for carotenoids in visible spectrophotometer. The amount of chlorophyll a and b, total chlorophyll and carotenoids were calculated according to Witham *et al.* (1971).

#### *Total protein amount and peroxidase enzyme activity analyzes*

The total protein amount and peroxidase activity were detected according to Bradford (1976) and, Herzog and Fahimi (1973) methods respectively. Harvested 1g fresh leaf samples were frozen in liquid nitrogen. The leaves were homogenized in tris-glycine buffer (pH 8.3) containing 1mM EDTA. The homogenates were centrifuged and the supernatants were used for enzyme activity and protein content assays. Total soluble protein content was determined to Bradford (1976) using bovine serum albumin as a standard. In the Bradford assay, the protein concentration is determined by quantifying Coomassie Brilliant Blue G-250 dye binding to an unknown protein solution compared to known standards.

The tubes containing 100 $\mu$ L aliquots of known concentrations of bovine serum albumin were prepared. Then, 1mL Coomassie Brilliant Blue solution was added to each tube and the mixtures vortexed. Reactions were incubated for 2min at room temperature. The absorbance at 595nm was determined against the blank, and a standard curve of absorbance versus protein concentration was plotted. Peroxidase (PO) activity was determined by Herzog and Fahimi (1973). The reaction mixture contained 3,3'-diaminobenzidinetetrahydrochloride dihydrate (DAB) solution containing 0.1% (w/v) gelatin, 150 mM Na-phosphate-citrate buffer (pH 4.4) and 0.6% H<sub>2</sub>O<sub>2</sub>. The absorbance increase at 465nm was monitored for 3 minutes. One enzyme unit is defined as  $\mu$ mol $\cdot$ mL<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> broken down per min.

#### *Proline amount analysis*

The modified method of Bates *et al.* (1973) was used to determine the proline content. The leaf samples were homogenized in 3% (w/v) sulfosalicylic acid solution and then centrifuged. The supernatant was taken in a test tube where glacial acetic acid and acid ninhydrin solution were added. The tubes were incubated for an hour in a boiling water bath and then allowed to cool to room temperature. After addition of cold toluene, the mixture was vortexed and allowed to stand to separate toluene and aqueous phase. The absorbance of the toluene phase was measured at 520nm with a spectrophotometer. The proline concentration was calculated from a proline standard curve and expressed as  $\mu$ mol/g FW.

#### *Total phenolic amount analysis*

A fresh sample ground from a 0.1g phenolic compound was extracted with 500  $\mu$ L 80 % aqueous methanol in boiling water bath (80°C) for 15 minutes and extracts were ultracentrifuged for 10 minutes at 500g, then the pellet was again extracted in the same procedure (Gayosa *et al.*, 2004). The total amount of phenolics was determined using the Folin-Ciocalteu assay (Singleton *et al.*, 1999). 100 $\mu$ L of extract was added 750 $\mu$ L of Folin-Ciocalteu phenol-reactive mixture and shaken. After 5 minutes, 750 $\mu$ L (6%) Na<sub>2</sub>CO<sub>3</sub> solution was added. After incubation at room temperature for 90 minutes, the absorbance against the prepared reagent blank was determined at 765nm with a UV-visible spectrophotometer. The total phenolic content was expressed in mg. Gallic acid was used as standard.

#### *Statistical analysis*

Experimental data were analyzed using the Tukey test at P < 0.05 level (1954).

## **Results and discussion**

#### *Growth measurements*

The effects of *P. falcata* extracts on the growth parameters of wild mustard are given in Tables (1,2). Reductions in ethanol (24.34%), ethyl acetate (24.34%), 25mg.mL<sup>-1</sup> *P. falcata* in ethanol (30.92%), 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate (6.25%) treatments in root length, and 25mg.mL<sup>-1</sup> *P. falcata*

in ethanol (16.35%), 50mg.mL<sup>-1</sup> *P. falcata* in ethanol (6.81%), 25mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate (18.66%), 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate (12.40%) in shoot length detected. All reductions in fresh and dry weight were determined in all treatments except the 25mg.mL<sup>-1</sup> *P. falcata* in distilled water at the shoot, in all treatments at the root. The increase in shoot fresh weight was 10.04%, shoot dry weight was 16.67%. Changes in growth parameters are not significant (p<0.05). *Arabidopsis thaliana* (L.) Heynh. root development has been found to be dose-dependent after treatment with different bibenzyls.

Isolated from the calli of *Marchantia paleacea* Bertol. (Wang *et al.*, 2013). Tsubota *et al.* (2006) observed *Sphagnum palustre* L., *Racomitrium japonicum* Dozy & Molk., *Dicranum japonicum* Mitt. and *Hypnum plumaeforme*.

Wils have allelopathic activity against the growth and development of lettuce seedlings. Similarly, *Rhynchosstegium pallidifolium* (Mitt.) A. Jaeger extracts had an inhibitory effect on the development of cress, lettuce and alfalfa (dicotyledonous), and ryegrass, timothy and *Digitaria sanguinalis* (L.) Scop. (monocotyledonous) (Kato-Noguchi, 2010).

**Table 1.** Effect of *Palustriella falcata* extracts at different concentrations and solvents on root length, fresh and dry weight of wild mustard. The same letters are not significantly different to the control at p<0.05 (Tukey test).

Treatments	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
a	3.040±0.764 <sup>x</sup>	0.0031±0.0016 <sup>x</sup>	0.0016±0.0014 <sup>x</sup>
b	2.300±0.908 <sup>x</sup>	0.0013±0.0008 <sup>x</sup>	0,0.0005±0.0001 <sup>x</sup>
c	2.300±0.758 <sup>x</sup>	0.0028±0.0012 <sup>x</sup>	0.0006±0.0004 <sup>x</sup>
d	3.150±0.991 <sup>x</sup>	0.0013±0.0012 <sup>x</sup>	0.0009±0.0003 <sup>x</sup>
e	3.100±0.953 <sup>x</sup>	0.0013±0.0012 <sup>x</sup>	0.0005±0.0002 <sup>x</sup>
f	2.100±0.660 <sup>x</sup>	0.0014±0.0002 <sup>x</sup>	0.0007±0.0005 <sup>x</sup>
g	3.086±0.672 <sup>x</sup>	0.0013±0.0008 <sup>x</sup>	0.0008±0.0006 <sup>x</sup>
h	3.429±1.321 <sup>x</sup>	0.0006±0.0004 <sup>x</sup>	0.0005±0.0003 <sup>x</sup>
i	2.850±0.733 <sup>x</sup>	0.0009±0.0007 <sup>x</sup>	0.0008±0.0006 <sup>x</sup>

a: control. b: ethanol. c: ethyl acetate. d: 25mg.mL<sup>-1</sup> *P. falcata* in distilled water. e: 50mg.mL<sup>-1</sup> *P. falcata* in distilled water. f: 25mg.mL<sup>-1</sup> *P. falcata* in ethanol. g: 50mg.mL<sup>-1</sup> *P. falcata* in ethanol. h: 25mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate. i: 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate.

**Table 2.** Effect of *Palustriella falcata* extracts at different concentrations and solvents on shoot length, fresh and dry weight of wild mustard. The same letters are not significantly different to the control at p<0.05 (Tukey test).

Treatments	Shoot length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
a	14.840±2.888 <sup>x</sup>	0.448±0.219 <sup>x</sup>	0.024±0.018 <sup>x</sup>
b	16.300±4.339 <sup>x</sup>	0.326±0.089 <sup>x</sup>	0.016±0.006 <sup>x</sup>
c	15.700±2.515 <sup>x</sup>	0.202±0.132 <sup>x</sup>	0.014±0.007 <sup>x</sup>
d	16.388±2.611 <sup>x</sup>	0.493±0.208 <sup>x</sup>	0.028±0.014 <sup>x</sup>
e	15.233±3.270 <sup>x</sup>	0.262±0.104 <sup>x</sup>	0.012±0.008 <sup>x</sup>
f	12.414±4.055 <sup>x</sup>	0.250±0.135 <sup>x</sup>	0.014±0.008 <sup>x</sup>
g	13.829±2.504 <sup>x</sup>	0.259±0.083 <sup>x</sup>	0.011±0.005 <sup>x</sup>
h	12.071±2.299 <sup>x</sup>	0.196±0.100 <sup>x</sup>	0.009±0.004 <sup>x</sup>
i	13.000±1.000 <sup>x</sup>	0.195±0.070 <sup>x</sup>	0.010±0.003 <sup>x</sup>

a: control. b: ethanol. c: ethyl acetate. d: 25mg.mL<sup>-1</sup> *P. falcata* in distilled water. e: 50mg.mL<sup>-1</sup> *P. falcata* in distilled water. f: 25mg.mL<sup>-1</sup> *P. falcata* in ethanol. g: 50mg.mL<sup>-1</sup> *P. falcata* in ethanol. h: 25mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate. i: 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate.

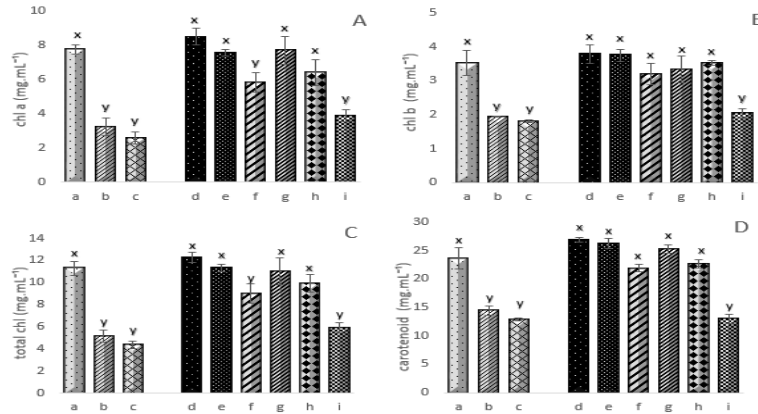
*Photosynthetic pigment amount analysis*

The effects of *P. falcata* extracts on the levels of chlorophyll *a* (Chla), chlorophyll *b* (Chlb), total Chlorophyll (total Chl) and carotenoid in wild mustard are shown in Fig. 1. The amount of Chla increased with the 25mg.mL<sup>-1</sup> *P. falcata* in distilled

water treatment, and decreased with the other treatments, compared to the control. The highest decrease was seen in ethyl acetate (66.72%) for all groups, and with 50mg.mL<sup>-1</sup> in ethyl acetate (49.90%) for the *P. falcata* treated groups. The Chlb and total Chl amounts increased in the 25 and

50mg.mL<sup>-1</sup> *P. falcata* distilled water treatments, and decreased with the other treatments, compared to the control. The highest decrease was observed in ethyl acetate (48.63% for Chlb; 61.06% for total Chl) for all groups, and 50mg.mL<sup>-1</sup> ethyl acetate (42.32% for Chlb; 47.53% for total Chl) for the *P. falcata* treated

groups (Fig. 1). It was found that the carotenoid levels increased in the 25–50mg.mL<sup>-1</sup> *P. falcata* distilled water, and 50mg.mL<sup>-1</sup> *P. falcata* ethanol treatments, and decreased in the other treatment groups, compared to the control. The biggest decrease of carotenoid levels was in ethyl acetate (45.47%; Fig. 1).

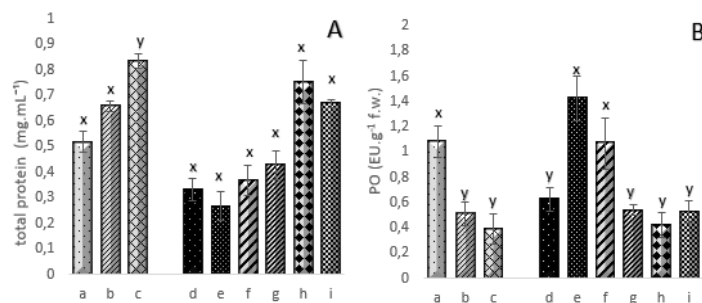


**Fig. 1.** Effect of *Palustriella falcata* extracts at different concentrations and solvents on the amount of photosynthetic pigments [Chla (A). Chlb (B). total Chl (C). carotenoids (D)] of wild mustard. Vertical bars represent Standard Error (n=3). The same letters are not significantly different to the control at p<0.05 (Tukey test). a: control. b: ethanol. c: ethyl acetate. d: 25mg.mL<sup>-1</sup> *P. falcata* in distilled water. e: 50mg.mL<sup>-1</sup> *P. falcata* in distilled water. f: 25mg.mL<sup>-1</sup> *P. falcata* in ethanol. g: 50mg.mL<sup>-1</sup> *P. falcata* in ethanol. h: 25mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate. i: 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate.

The harmful effects of allelochemicals are considered as allelochemical stress, and this decreases the amount of Chla, Chlb, and carotenoids of recipient plants (Cruz-Ortega *et al.*, 2002; Singh *et al.*, 2009).

*Total protein amount and peroxidase enzyme activity analyzes*

Total protein amount and peroxidase activity of wild mustard plants are shown in Fig. 2.



**Fig. 2.** Effects of *Palustriella falcata* extracts at different concentrations and solvents on the amount of total protein (A) and activity of peroxidase (B) of wild mustard. Vertical bars represent Standard Error (n=3). The same letters are not significantly different to the control at p<0.05 (Tukey test).

a: control. b: ethanol. c: ethyl acetate. d: 25mg.mL<sup>-1</sup> *P. falcata* in distilled water. e: 50mg.mL<sup>-1</sup> *P. falcata* in distilled water. f: 25mg.mL<sup>-1</sup> *P. falcata* in ethanol. g: 50mg.mL<sup>-1</sup> *P. falcata* in ethanol. h: 25mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate. i: 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate.

In the current study, the total protein amounts increased in the ethanol, ethyl acetate, 25 and 50mg.mL<sup>-1</sup> *P. falcata* ethyl acetate treatments, and decreased in other treatment groups, compared to the control. The highest increase was in ethyl acetate (60.18%) for all groups, and with 25mg.mL<sup>-1</sup> in ethyl acetate (44.25%) for the *P. falcata* treated groups (Fig. 2A).

Plants use normal cell proteins such as phenol biosynthesis enzymes, hydrolases, enzyme inhibitors, structural proteins, molecular chaperones, etc. and specific stress proteins against stress conditions (Ozen and Onay, 2013). We think that synthesis of new sets of proteins which are specific to the particular type of stress may have been realized especially in ethanol, ethyl acetate, 25mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate, 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate treatment groups.

In the peroxidase activity, the most significant increase is in 50mg.mL<sup>-1</sup> *P. falcata* in distilled water (31.49%), and decreases are in ethyl acetate (64.09%)

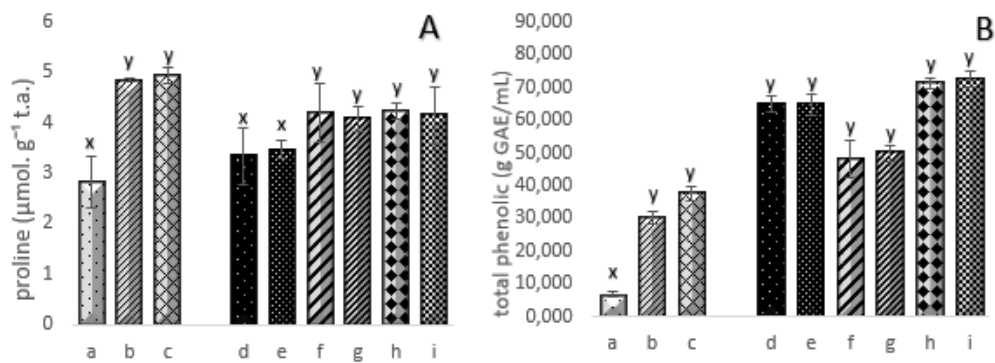
and 25 mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate (60.94%) respectively (Fig. 2B).

Non-specific peroxidase (PO, EC 1.11.1.7) play an important role in the antioxidative protection. The amount of this enzyme is usually increased under stress conditions. In the study, PO activity increased only in 50mg.mL<sup>-1</sup> *P. falcata* in distilled water. The decrease in peroxidase activity in other groups suggests that the plant uses a different pathway as its defense mechanism.

*Proline and Total phenolic amount analyzes*

Increases in the amount of proline and total phenolics were determined in all treatment groups (Fig. 3). In the *P. falcata* treated groups, the highest increase in the proline amount (49.82%) was seen with the 25mg.mL<sup>-1</sup> *P. falcata* ethyl acetate treatment.

The biggest increase in the total phenolic amount (1050.76 %) was seen with the 50 mg.mL<sup>-1</sup> *P. falcata* ethyl acetate treatment (Fig. 3).



**Fig. 3.** Effects of *Palustriella falcata* extracts at different concentrations and solvents on the amount of proline (A) and total phenolic (B) of wild mustard. Vertical bars represent Standard Error (n=3). Same letters are not significantly different to the control at p<0.05 (Tukey test).

a: control. b: ethanol. c: ethyl acetate. d: 25mg.mL<sup>-1</sup> *P. falcata* in distilled water. e: 50mg.mL<sup>-1</sup> *P. falcata* in distilled water. f: 25mg.mL<sup>-1</sup> *P. falcata* in ethanol. g: 50mg.mL<sup>-1</sup> *P. falcata* in ethanol. h: 25mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate. i: 50mg.mL<sup>-1</sup> *P. falcata* in ethyl acetate.

Proline and phenolic compounds are synthesized by plants in response to abiotic stress (e.g. UV damage, drought, freezing), and biotic stress (e.g. microbial, allelochemical, insect attacks) (Xie and Lou, 2009; Bhattacharya *et al.*, 2010; Turkyilmaz Unal, 2013).

Also, phenolic compounds play an important role in the interaction of plants with their environment. Phenolics may act as signals between plants and symbiotic or pathogenic organisms. It represents the main allelopathic compounds that alter the floristic

composition in the plant community, and affect seed germination, seedling growth and other biochemical and physiological processes (Harborne, 1993, Fritz *et al.*, 2007). The decrease in photosynthetic pigment and total protein amount, and the increase in levels of proline and total phenolics are thought to be due to the biotic stress created by the *P. falcata*. Thakur and Kapila (2015) suggested that some liverworts may be used for biological control of the *Bidens pilosa* L. weeds, due to their inhibitory effect on germination and seedling growth.

In light of the data obtained, it is thought that the changes observed were due to the allelopathic effect of the *P. falcata* plant. The decrease in growth parameters and photosynthetic pigment amounts, the increase in proline and phenolic amounts indicate that the wild mustard was adversely affected by the *P. falcata*. The low peroxidase enzyme activity suggests that the plant prefer to increase the proline and phenolic compounds in the defense mechanism. It was determined that ethyl alcohol and ethyl acetate were more effective when used as a solvent. This study has provided preliminary data to support the use of bryophytes in the biological control of weeds. Future studies requires the use of different solvents, concentrations and analyzes. Investigations into transferring these findings to the control of common weeds in agricultural areas will be carried out in future studies.

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