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Phytochemical composition and biopesticidal potential of *Gypsophila pilosa* Huds

Yusuf Bayar^{1*} , Melih Yılar¹ , Hayriye Didem Sağlam Altinköy¹ and Abdurrahman Onaran²

Abstract

Background *Gypsophila* species are rich in saponins and phenolic compounds that exhibit antibacterial, cytotoxic, and antioxidant activities; however, there is not much information about their herbicidal, insecticidal, or fungicidal effects.

Result This study was to investigate the phytochemical composition and examine the herbicidal, antifungal, and insecticidal effects of *Gypsophila pilosa* Huds., a species of the Caryophyllaceae family, to evaluate its potential as an environmental friendly biopesticide for sustainable agriculture. The soil properties at the collection site were evaluated, and plant material was collected from Kırşehir Province, Turkey. The phytochemical structure of the aerial parts (leaves+flowers+shoots) methanol extract was analysed using LC-ESI-MS/MS. A phytochemical study found eight phenolic compounds, with rosmarinic acid (17,710 mg/kg), ferulic acid (13,955 mg/kg), and rutin (2,994 mg/kg) being the main components. Methanol and hexane extracts were prepared and tested for their herbicidal activity against *Rumex crispus* L., *Taraxacum officinale* F.H. Wigg. and *Triticum aestivum* L., as well as their antifungal activity against *Rhizoctonia solani* Kühn., *Sclerotinia sclerotiorum* (Lib.) de Bary, *Phytophthora infestans* (Mont.) de Bary and *Monilinia fructigena* (Pers.) Honey. Additionally, they were tested for their insecticidal activity against *Sitophilus granaries*. Herbicidal research indicated a significant decrease of germination and seedling development in *R. crispus* and *T. officinale*, with methanol extracts exhibiting total suppression of *T. officinale* at increased treatments. Antifungal assays showed that both methanol and hexane extracts inhibited the mycelial growth of *R. solani*, *P. infestans*, and *M. fructigena*, although *S. sclerotiorum* showed resistance. Insecticidal experiments demonstrated a dose- and time-dependent mortality of *S. granarius*, with the methanol extract obtaining a mortality rate of up to 61% at a 20% concentration after 96 h.

Conclusions This research is the first demonstration of the biopesticidal properties of *G. pilosa*, showing significant herbicidal, antifungal, and insecticidal effects. The findings indicate that *G. pilosa* could be used as an environmental friendly alternative for chemical pesticides, improving integrated pest management strategies while supporting sustainable farming practices.

Keywords *Gypsophila pilosa*, Antifungal activity, Herbicidal activity, Insecticidal activity, Biopesticide

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Background

As humans began sedentary life, they started agricultural practices. These days, both field and greenhouse settings are used for intensive production. Production in organic agriculture has been limited, and the most common production strategy is conventional. Even though there are only so many things that can be produced in each region using conventional methods, the number of people living below the poverty line rises as the world's population grows. The world's most grown crops include fruits, vegetables, cereals, and legumes [1]. The production of fruits, vegetables, legumes, and cereals is restricted by significant biotic factors. The three main ones are insects, plant diseases, and weeds. *Sitophilus* species are major pests of stored grains, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* damage vegetables and fruits, *Monilinia fructigena* target fruits, while *Phytophthora infestans* (Mont.) de Bary and *Rumex crispus* cause significant damage on potatoes.

Chemical pesticides are primarily used to control these pests; however, their applications are restricted due to the negative effects on the environment, humans, and non-target organisms, while biopesticide treatments are increasingly being used as an alternative.

Plants have pesticidal features due to the presence of alkaloids, terpenes, and secondary metabolites. Currently, it has been shown that over 2,500 plants have biopesticide traits [2]. Research has demonstrated that plants from the Asteraceae, Lamiaceae, Brassicaceae, and Caryophyllaceae families showed fungicidal, acaricidal, insecticidal, and herbicidal traits [3–7]. The family of Caryophyllaceae has a great diversity with about 80 genera, 2,100 species, spreading in the warm and temperate regions of the Northern Hemisphere, in the South Hemisphere and in the Mediterranean. The genus *Gypsophila* has 150 species and is spread in the Asia, Australia, Europe, North America and part of Africa [8]. *Gypsophila* species in Turkey have a vertical distribution from 100 m to 2,800 m and have a horizontal distribution in most Iran-Turan phytogeographic regions [9]. It is reported that the soils where *Gypsophila* taxa grow are generally loamy, salt-free, and slightly alkaline in character, ranging from slightly to highly calcareous, low in phosphorus, high in potassium, and moderately organic in content. *Gypsophila pilosa* is a one-year-old plant that can grow up to 1 m in cultivated fields, roadsides, steppes, rarely off the coast of the Pinus Forest and on dune soils. This plant, which blooms in May, June, July, is known as oily grass, Çöven grass [9].

Gypsophila species are contained saponin and phenolic components [10, 11]. It has been demonstrated by different researchers that these compounds exhibit different biological activities such as antimicrobial, cytotoxic and antioxidants [8, 10]. Although studies conducted on

Gypsophila pilosa are very limited, the existing research primarily focuses on the determination of its antioxidant activity. However, no data on herbicidal, insecticidal, and fungicidal activity have been found before. In this study, the biological study of *G. pilosa* plant extract, which is spreading naturally in Kirsehir Province, on different plant diseases, weeds and insect was determined.

Materials and methods

Plant material

Plant materials of *Gypsophila pilosa* Huds. were obtained from the campus of Kirsehir Ahi Evran University during the flowering period of 2018–2019. This plant material was collected by Melih YILAR and identified at the Herbology Laboratory of the Plant Protection Department, Faculty of Agriculture, Kirsehir Ahi Evran University. The plant species (Accession number: MY-1000) is kept in the Herbology Laboratory. Plants which are used in the experiment were not endemic and no need to permissions. The harvested plant material was dried at room temperature and ground using an electronic grinder. It was stored at room temperature in a jar until used in the experiment.

Methanol and hexane extracts

The methanol and hexane extracts were made using the same technique. 100 g of ground *G. pilosa* was added to a 1 L beaker and filled with 600 ml of methanol or hexane. The solutions were kept in the shaker at room temperature for 24 h. After the extraction, the resulting solution had been filtered by filter paper. The methanol or hexane in the solution were evaporated until solid materials remained at 32 °C using an evaporator. The remaining solids were diluted with acetone (99.6% pure) or distilled water, so the solution was accepted as a stock solution. The stock solution was kept at 4 °C until used in the experiments.

Reagents and solutions

All the solutions were made with ultra-pure water and analytical-grade chemicals (Milli-Q, Millipore, Bedford, MA, USA). For the analysis of specific phenolic compounds, Merck (Darmstadt, Germany) provided methanol and chloroform of LC-MS grade, while J.T. Baker (Phillipsburg, NJ, USA) provided formic acid of HPLC grade. The ultra-pure individual phenolic standards (all with purity ≥ 95%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and included ferulic acid, p-coumaric acid, vanillin, rutin, (+)-catechin, caffeic acid, (–)-epicatechin, salicylic acid, gallic acid, p-hydroxybenzoic acid, resveratrol, oleuropein, rosmarinic acid, taxifolin, protocatechuic acid, protocatechuic aldehyde, and ellagic acid. Each chemical was produced in 1000 mg/L stock standard solutions in methanol and kept at 4 ± 2 °C.

External standard calibration has been used to assay the phenolic chemicals that are being studied.

Identification of phenolic compounds in methanol extract by LC-ESI-MS/MS

The extraction of free individual phenolic compounds was conducted using modifications of the method established by Da Silva et al. [12]. A volumetric flask (10 mL) containing 3.0 ± 0.1 g of plant material was used for each sample. The volume was then adjusted using an 80:20 v/v methanol/chloroform solution. Following the transfer of the resultant solution to a polyethylene tube, the extraction was carried out for one hour at room temperature in an ultrasonic bath (KUDOS, Turkey) and then centrifuged for ten minutes at $1500 \times g$ (SIGMA, Germany). After passing through a $0.45 \mu\text{m}$ PVDF syringe filter, an aliquot of the supernatant was transferred straight into a vial and then injected into an LCMS/MS apparatus. According to Seraglio et al. [13], the sample supernatant was examined, and the various phenolics were identified using LC-MS/MS (liquid chromatography-tunnel mass spectrometry). The findings were reported in dry matter (DM) mg kg^{-1} . The Access MAX triple quadrupole mass spectrometer (Thermo Fisher Scientific, Germany) with an electrospray ionisation source (ESI) was used in conjunction with a Thermo Scientific TSQ Quantum Access Max chromatographic system (Thermo Fisher Scientific, Germany). A Thermo Scientific ODS HYPERSIL C18 column ($250 \text{ mm} \times 4.6 \text{ mm}$; $5 \mu\text{m}$ particle diameter) was used to accomplish the chromatographic separation. The injection volume was $20 \mu\text{L}$, and the flow rate was $700 \mu\text{L}$ per minute. The separation was conducted at 30°C employing gradient elution as detailed below: 100% solvent A (v/v) from 0 to 1.0 min, 100% to 5.0% solvent A

(v/v) from 1.0 to 22.0 min, 5% solvent A (v/v) from 22.0 to 25.0 min, 5.0% to 0.0% solvent A (v/v) from 25.0 to 30.0 min, and 0.0% to 100% solvent A (v/v) from 30.0 to 34.0 min. The mass spectrometry analysis utilised an ion source operating in both positive and negative ionisation modes, configured with the following parameters: ion spray (IS) voltage at 4000 V; curtain gas at 25 psi; nebuliser gas (GS1) and auxiliary gas (GS2) at 30 psi; and a source temperature of 300°C . Nitrogen served as a nebulising and collision gas.

Multiple reaction monitoring (SRM) mode was employed for the acquisition, and data processing and acquisition were carried out using the Xcalibur software (Thermo Fisher Scientific, Germany) [13]. According to Seraglio et al. [13], the mass spectrometry parameters used to identify and quantify the different phenolics are listed in Table 1. The calibration curves, detection limits, and quantification limits are detailed in Table 2.

Herbicidal activities

This experiment assessed on test plants (*Triticum aestivum* L., *Rumex crispus* L. and *Taraxacum officinale* F.H. Wigg.). Seeds from test plants were uniformly distributed (25 pieces) on two layers of filter paper within 9 cm petri dishes. Three different doses (1000, 2000, and 3000 ppm) were made from the stock solution, with pure water providing the control. Five ml solutions from various dosages were given to seeds on the Petri dishes. The Petri dishes were incubated at 25°C for 12 h in darkness, followed by 12 h of light, for a duration of 4 weeks. After the incubation period concluded, the germination rate, roots, and shoot lengths of the test seeds were assessed. The experiment included four repetitions and was performed twice [14].

Table 1 Mass spectrometry parameters for SRM transitions of phenolic compounds in the negative and positive ion mode monitoring

Name	Parent	Product	CE	Polarity	Name	Parent	Product	CE	Polarity
Salicylic Acid	137.14	65.51	35	--	Taxifolin	303.0	126.2	23	-
		93.26	18	-			285.5	15	-
Rutin	609.37	300.6	38	-	Resveratrol	228.98	107.2	22	+
		301.7	34	-			135.1	14	+
p-hydroxybenzoic acid	137.9	66.6	38	-	Catechin	289.2	203.9	22	+
		94.6	17	-			245.7	17	+
Ellagic Acid	300.9	229.7	28	-	Epicatechin	291.5	123.3	15	+
Protocatechuic Acid	153.808	110.4	17	-			139.3	16	+
		92.5	27	-	protocatechuic aldehyde	136.9	92.25	25	-
p-coumaric acid	163.9	94.3	33	-			108.2	25	-
		120.2	17	-	Vannilin	150.91	92.3	23	-
Oleuropein	539.1	275.8	22	-			136.1	16	-
		377.5	16	-	Rosmarinic Acid	359.18	134.3	44	-
Gallic Acid	169.7	80.5	25	-			162.2	20	-
		126.2	16	-	Ferulic Acid	193.35	134.1	17	-
Caffeic acid	179.7	135.2	27	-			178	15	-
		136.2	18	-					

Table 2 Analytical parameters of LC-ESI-MS/MS quantitative method for determination of phenolic acids

Phenolic acids	LOD ^a	LOQ ^b	Regression Coefficient (R ²)	Equation
Gallic Acid	0.061	0.203	0.997	Y = 602,478*X
Protocatechuic Acid	0.049	0.162	0.995	Y = 346,483*X
Protocatechuic aldehyde	0.026	0.087	0.995	Y = 2.56226e + 006*X
Catechin	0.068	0.227	0.998	Y = 5.23694e + 007*X
Epicatechin	0.045	0.151	0.999	Y = 4.63426e + 007*X
Caffeic acid	0.047	0.157	0.996	Y = 5.58071e + 006*X
Vanillin	0.023	0.076	0.999	Y = 1.64619e + 006*X
Taxifolin	0.058	0.194	0.997	Y = 1.07631e + 007*X
p-coumaric acid	0.116	0.387	0.997	Y = 158,306*X
Ferulic Acid	0.061	0.204	0.997	Y = 236,062*X
Rosmarinic Acid	0.029	0.095	0.995	Y = 1.35984e + 007*X
Oleuropein	0.050	0.167	0.996	Y = 7.99343e + 006*X
p-hydroxybenzoic acid	0.031	0.104	0.998	Y = 1.12917e + 007*X
Salicylic Acid	0.030	0.099	0.999	Y = 1.22396e + 007*X
Rutin	0.007	0.024	0.995	Y = 4.97449e + 007*X
Resveratrol	0.030	0.099	0.999	Y = 2.04534e + 007*X
Ellagic Acid	0.087	0.289	0.996	Y = 1.11394e + 007*X

^aLimit of detection, ^blimit of quantification

Antifungal activities

This study examined *Monilinia fructigena* (Pers.) Honey, *Phytophthora infestans* (Mont.) de Bary, *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Rhizoctonia solani* Kühn. The aforementioned solutions have been prepared in PDA at dosages of 1500, 3000, and 4500 ppm. The PDA media was cooled to the temperature that was between 45 and 50 °C [15]. Ten ml of PDA media were put into a 60 mm petri dish. The fungus was added plain as a negative control. A fungicide (Thiram) was used as a positive control. Mycelium discs with a diameter of 5 mm, obtained from confirmed plant pathogen cultures, were inoculated in petri dishes containing PDA media and treated with extracts for 7–10 days in the trials. Fungal cultures were grown for 7 days at 25 ± 1 °C following inoculation. This experiment was conducted with four repetitions and repeated twice. The diameters of the emerging mycelium in the petri dishes were measured using a digital calliper.

The following formula was used to calculate the percentage of mycelium development inhibition in the extracts:

$$I: \frac{(dc-dt)}{dc} \times 100$$

I: Percent mycelium development inhibition rate.

dc: Development of mycelium in control.

dt: Development of mycelium in the experiment [16].

Insecticidal activities

Sitophilus granarius L. (Coleoptera: Curculionidae) was reared in an incubator at 25 ± 1 °C and 60 ± 5% humidity, in complete darkness, on whole wheat in a 1-liter jar. Adults were transferred to one-third wheat grains in the jar and kept there for one week. The adults were afterwards separated from the jar and awaited the emergence of fresh adults. An adult insect no older than one week was used in the experiment. Three different doses (5%, 10%, and 20%) were prepared by acetone prepared from the stock solution, with acetone used as the control. 1 µl of each extract was topically applied to the dorsal surface of each insect using a micro syringe. After treatment, insects were transferred to a 9 cm petri dish with 1 g of whole wheat grains. The experiment was carried out using a completely randomised method. Each replication had ten adult insects, with the experiment being carried out in five repetitions and repeated twice. The insects were touched with a needle under a stereomicroscope to determine the mortality, and they were then watched for a while. With no signs of movement, they were considered dead. Mortality was recorded at 24, 48, 72, and 96 h.

Data analysis

The experiment assessed the severity differences between treatments by variance analysis (ANOVA), and averages were compared using the DUNCAN test. The SPSS software (version 29) was used to perform statistical analyses.

Results

Phytochemical Composition of *Gypsophila pilosa*

The phenolic profile of *Gypsophila pilosa* extract indicates that although the overall phenolic diversity is limited, several compounds are present at notable levels. According to the HPLC/MS analysis, rosmarinic acid (17.710 mg/kg), ferulic acid (13.955 mg/kg), and rutin (2.994 mg/kg) were identified as the major constituents, while epicatechin (0.371 mg/kg), caffeic acid (1.329 mg/kg), vanillin (2.916 mg/kg), p-hydroxybenzoic acid (0.455 mg/kg), and salicylic acid (1.020 mg/kg) were also detected in the plant extract (Table 3). Among these, rosmarinic acid and ferulic acid were found in the highest concentrations, suggesting that they are the primary contributors to the antioxidant capacity of the extract. In contrast, phenolic compounds such as gallic acid, protocatechuic acid, protocatechuic aldehyde, catechin, taxifolin, p-coumaric acid, oleuropein, resveratrol, and ellagic acid remained below the limit of detection (LOD) and were not quantified in the extract (Table 3). This indicates that the phenolic structure of *Gypsophila pilosa* tends to be concentrated around specific compounds rather than broadly distributed across diverse phenolic groups.

Table 3 The contents (mg kg⁻¹ dry matter) of phenolic compounds of *Gypsophila pilosa* methanol extract

RT	Phenolic Compound	mg Phenolic Compounds / Kg <i>Gypsophila pilosa</i>
10.05	Gallic Acid	< LOD
12.13	Protocatechuic Acid	< LOD
13.16	Protocatechuic aldehyde	< LOD
13.24	Catechin	< LOD
14.72	Epicatechin	0.371 ± 0.035
15.27	Caffeic Acid	1.329 ± 0.124
15.87	Vannilin	2.916 ± 0.331
16.68	Taxifolin	< LOD
17.00	p-coumaric acid	< LOD
17.19	Ferulic Acid	13.955 ± 0.321
17.82	Rosmarinic Acid	17.710 ± 0.754
18.00	Oleuropein	< LOD
18.12	p-hydroxybenzoic Acid	0.455 ± 0.036
18.13	Salicylic Acid	1.020 ± 0.076
18.26	Rutin	2.994 ± 0.036
18.45	Resveratrol	< LOD
19.47	Ellagic Acid	< LOD

*Means in same letter were not significantly different by ANOVA ($p < 0.05$), (LOD: limit of detection)

Overall, the findings demonstrate that *Gypsophila pilosa* is particularly rich in rosmarinic acid and ferulic acid, and the presence of these compounds provides a valuable basis for future studies investigating the plant's potential antioxidant, anti-inflammatory, or other biological activities.

Herbicidal activities

The germination and seed development of *Rumex crispus*, *Taraxacum officinale* and *Triticum aestivum* have been found to be herbicidally affected by the methanol and hexane extracts of *G. pilosa*. This effect has been demonstrated as statistically significant. However, variations in the effects were observed according to the extract and dosage for plant testing. The hexane extracts exhibited the most significant impact on seed germination in *T. aestivum* and *R. crispus*, whereas the methanol extracts demonstrated the maximum efficacy on *T. officinale* at 3000 ppm. The methanol extract suppressed the germination of *T. officinale* by 73.44%, 100%, and 100%, respectively. The hexane extract exhibited inhibition rates of 76.56%, 84.37%, and 93.74%, respectively. The methanol extract of *R. crispus* suppressed germination by 17.56%, 33.77%, and 35.13%, while the hexane extract resulted in inhibition rates of 16.21%, 28.37%, and 31.07% (Fig. 1). Both methanol and hexane extracts shown reduced efficacy in inhibiting germination in *T. aestivum*. *T. aestivum* showed greater tolerance compared to *R. crispus* and *T. officinale*. The methanol solution of *T. aestivum* presented inhibition rates of 2.66%, 6.66%, and 10.66%, while the hexane extracts demonstrated inhibition rates of 6.66%, 10.66%, and 14.66%, respectively (Fig. 1).

The development of seedlings has shown similar results to those observed in plant seed germination because of this investigation. The lowest and maximum concentrations of plant methanol and hexane extracts, respectively, both reduced the growth of *T. aestivum* roots and shoots. The hexane extract results were 97.74% to 94.54%, while the methanol extract results were 96.42% to 92.51% (Fig. 2).

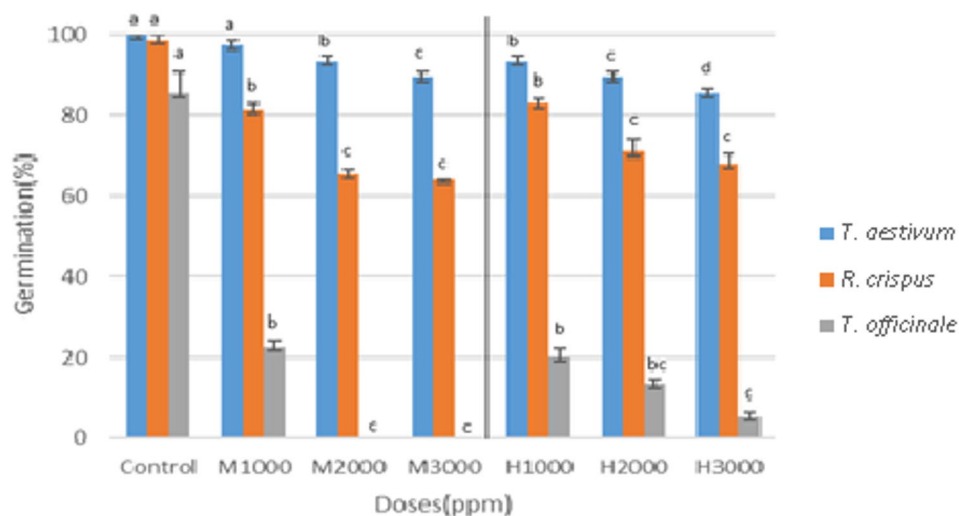


Fig. 1 The effect of *Gypsophila pilosa* extracts on *Rumex crispus*, *Taraxacum officinale* and *Triticum aestivum*. *Means in same letter were not significantly different by ANOVA ($p < 0.05$)

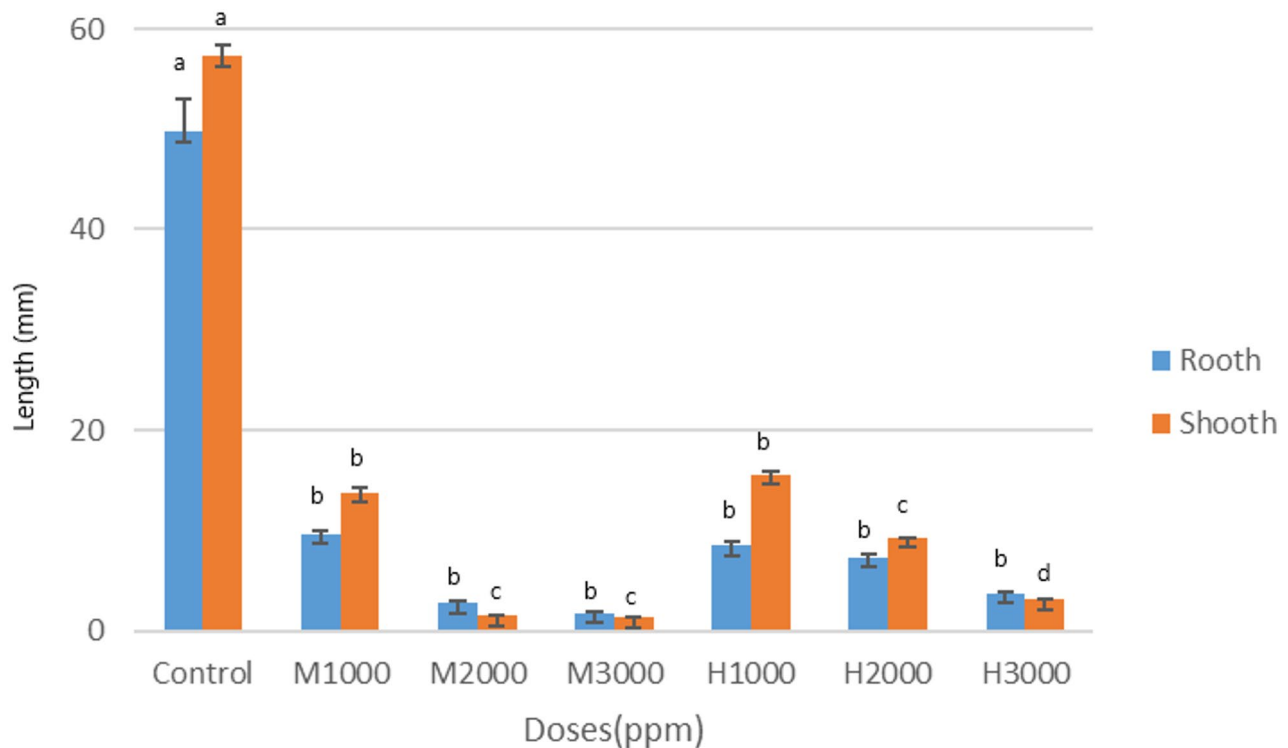


Fig. 2 The effect of *Gypsophila pilosa* extracts on *Triticum aestivum* shoot and roots development. *Means in same letter were not significantly different by ANOVA ($p < 0.05$)

Rumex crispus seedling development was affected at different levels depending on the extracts and application doses. This effect was found to be statistically significant. The methanol extracts were inhibited to roots at % 94.66%, 97.39%, 98.7% and shoots at 81%, 91.35%, %93.28% (Fig. 3).

Gypsophila pilosa extracts of hexane and methanol were found a higher phytotoxic effect on *T. officinale*. The methanol extracts of *G. pilosa* of 2000 and 3000 ppm doses completely inhibited the development of root and shoot of *T. officinale*. The hexane extracts was inhibited to root 40.76%, 86.01%, 98.61% and shoot %43.68%, 65.14%, %85.53% respectively (Fig. 4).

Antifungal activities

The antifungal activity values of the hexane extract obtained from *G. pilosa* against *R. solani*, *S. sclerotiorum*, *P. infestans* and *M. fructigena* were given in Table 4. The plant pathogens were showed different effectiveness against hexane extracts. The hexane extract prevented the mycelium development of *R. solani*, *M. fructigena* and *P. infestans* by 16.62%, 25.30% and 27.62% respectively compared to control. However, *S. sclerotiorum* has not had any effect on the development of mycelium. As a result of this hexane extracts experiment, the most sensitive pathogen was found *P. infestans*, while the most resistance was *S. sclerotiorum*.

Methanol extract *R. solani*, *S. sclerotiorum*, *P. infestans* and *M. fructigena* have been found to show differences in mycelium development depending on dose compared to control (Table 5). The extract inhibited mycelial growth of *M. fructigena*, *P. infestans* and *R. solani* by 17.60%, 27.31% and 30.72%, respectively, compared to control. However, as with hexane extract, *S. sclerotiorum* did not show any effect on mycelium growth.

Methanol extracts of *Gypsophila pilosa* have been found to inhibit mycelium development more than hexane extract on *P. infestans* and *R. solani* but however, it has been observed that the hexane extract affects the mycelium development of *M. fructigena* more. Despite these results, in both extracts, it was determined that *S. sclerotiorum* was not effective in the development of mycelium.

Insecticidal activities

This study assessed the insecticidal efficacy of methanol and hexane extracts obtained from *G. pilosa* on *S. graminis* at different dosages (5%, 10%, and 20%) and exposure durations (24, 48, 72, and 96 h). The results indicated that both extracts significantly raised mortality rates in a dose and time dependent manner (Fig. 5).

The methanol extract showed higher mortality rates than the hexane extract throughout all dosages and period. The highest mortality rate, 61%, was recorded at

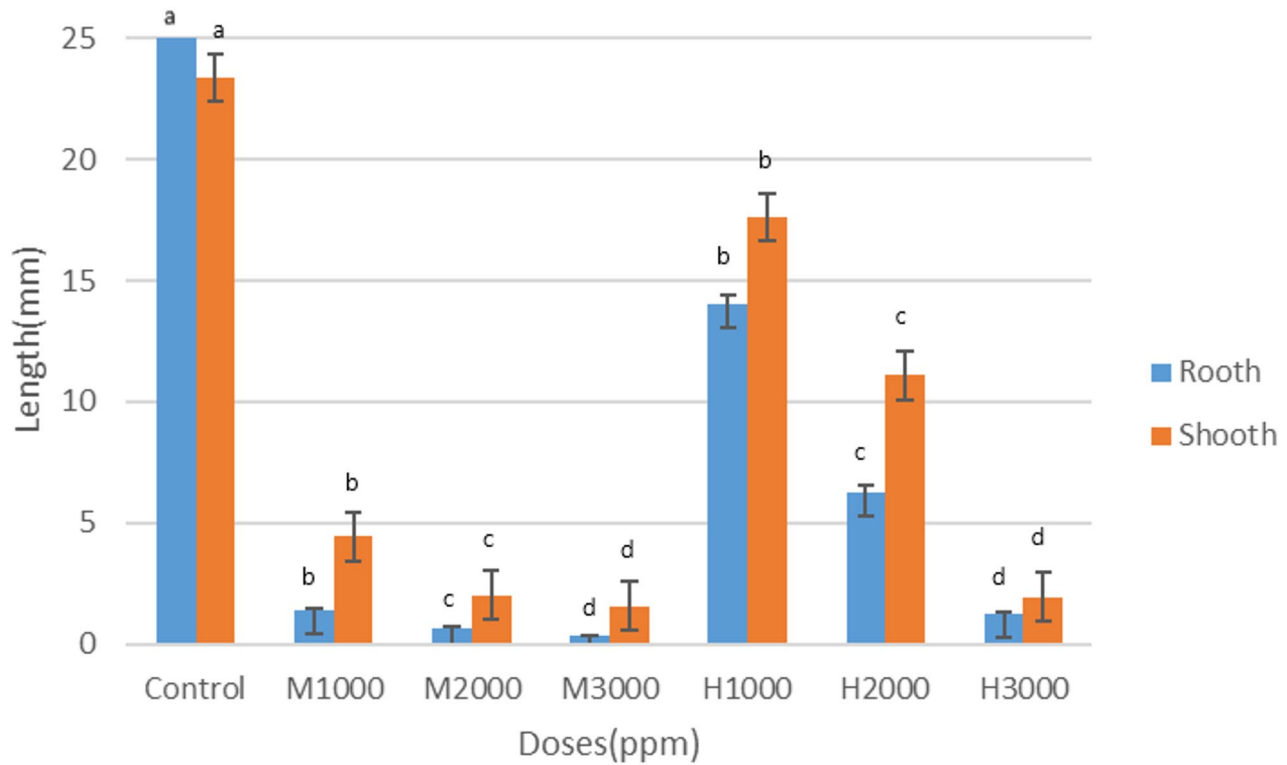


Fig. 3 The effect of *Gypsophila pilosa* extracts on *R. crispus* shoot and roots development. *Means in same letter were not significantly different by ANOVA ($p < 0.05$)

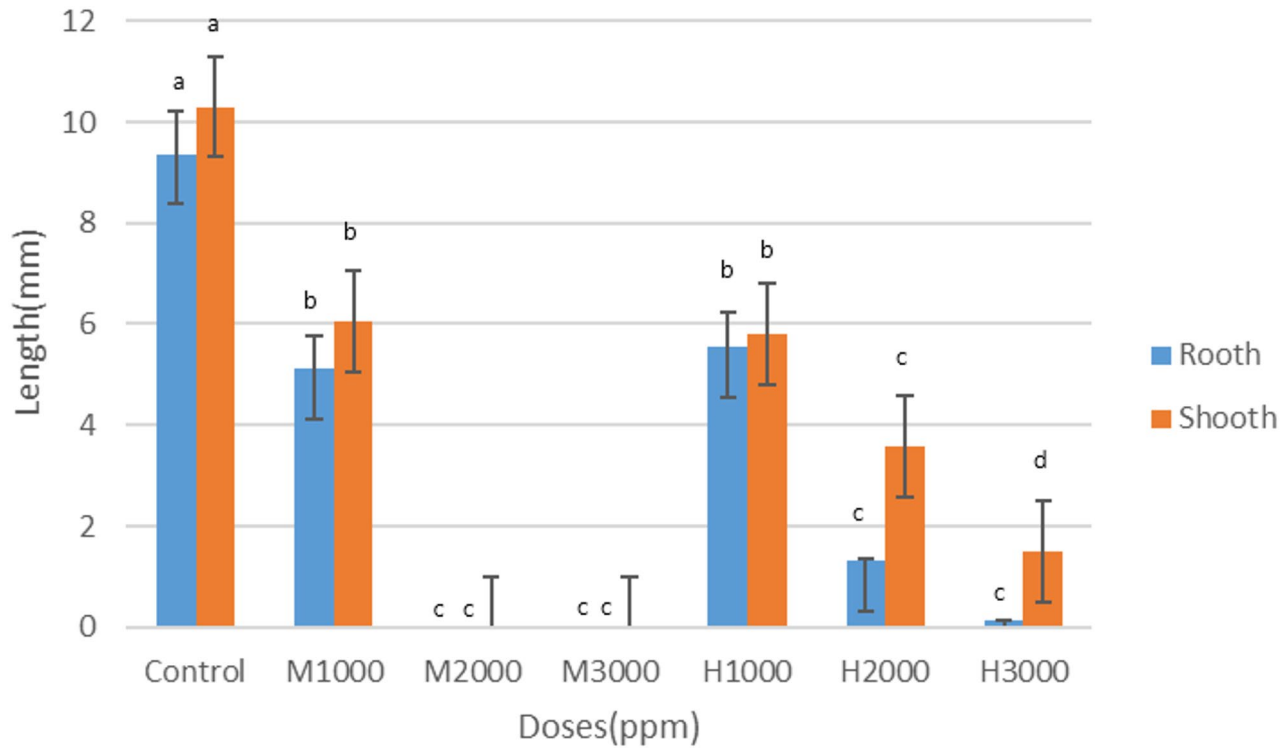


Fig. 4 The effect of *Gypsophila pilosa* extracts on *T. officinale* shoot and roots development. *Means in same letter were not significantly different by ANOVA ($p < 0.05$)

Table 4 Antifungal effects (%) of *Gypsophila pilosa* hexane extract

Doses (ppm)	<i>R. solani</i>	<i>S. sclerotiorum</i>	<i>P. infestans</i>	<i>M. fructigena</i>
Control ⁺	100 ± 0.00 ^{a*}	100 ± 0.00 ^a	100 ± 0.00 ^{a*}	100 ± 0.00 ^{a*}
Control ⁻	0.00 ± 0.00 ^d	0.00 ± 0.00 ^b	0.00 ± 0.00 ^d	0.00 ± 0.00 ^c
1500	0.00 ± 0.00 ^d	0.00 ± 0.00 ^b	17.72 ± 2.35 ^c	0.00 ± 0.00 ^c
3000	6.18 ± 3.23 ^c	0.00 ± 0.00 ^b	24.97 ± 0.83 ^b	0.00 ± 0.00 ^c
4500	16.62 ± 0.41 ^b	0.00 ± 0.00 ^b	27.62 ± 1.32 ^b	25.30 ± 0.96 ^b

*Means in the same column with the same letter were not significantly different by ANOVA ($p < 0.05$). Control⁺: Positive control; Control⁻: Negative control

Table 5 Antifungal effects (%) of *Gypsophila pilosa* methanol extract

Doses (ppm)	<i>R. solani</i>	<i>S. sclerotiorum</i>	<i>P. infestans</i>	<i>M. fructigena</i>
Control ⁺	100 ± 0.00 ^{a*}	100 ± 0.00 ^a	100 ± 0.00 ^{a*}	100 ± 0.00 ^{a*}
Control ⁻	0.00 ± 0.00 ^d	0.00 ± 0.00 ^b	0.00 ± 0.00 ^d	0.00 ± 0.00 ^c
1500	10.72 ± 5.39 ^{cd}	0.00 ± 0.00 ^b	8.63 ± 8.18 ^d	0.00 ± 0.00 ^c
3000	19.31 ± 3.98 ^{bc}	0.00 ± 0.00 ^b	25.73 ± 2.18 ^c	0.00 ± 0.00 ^c
4500	27.31 ± 2.96 ^b	0.00 ± 0.00 ^b	30.72 ± 2.28 ^b	17.60 ± 8.52 ^b

*Means in the same column with the same letter were not significantly different by ANOVA ($p < 0.05$). Control⁺: Positive control; Control⁻: Negative control

96 h with a 20% methanol extract. However, the hexane extract under same conditions caused 49% mortality. Statistical analysis showed that the differences between methanol and hexane extracts at comparable doses and durations were significant ($p < 0.05$).

Discussion

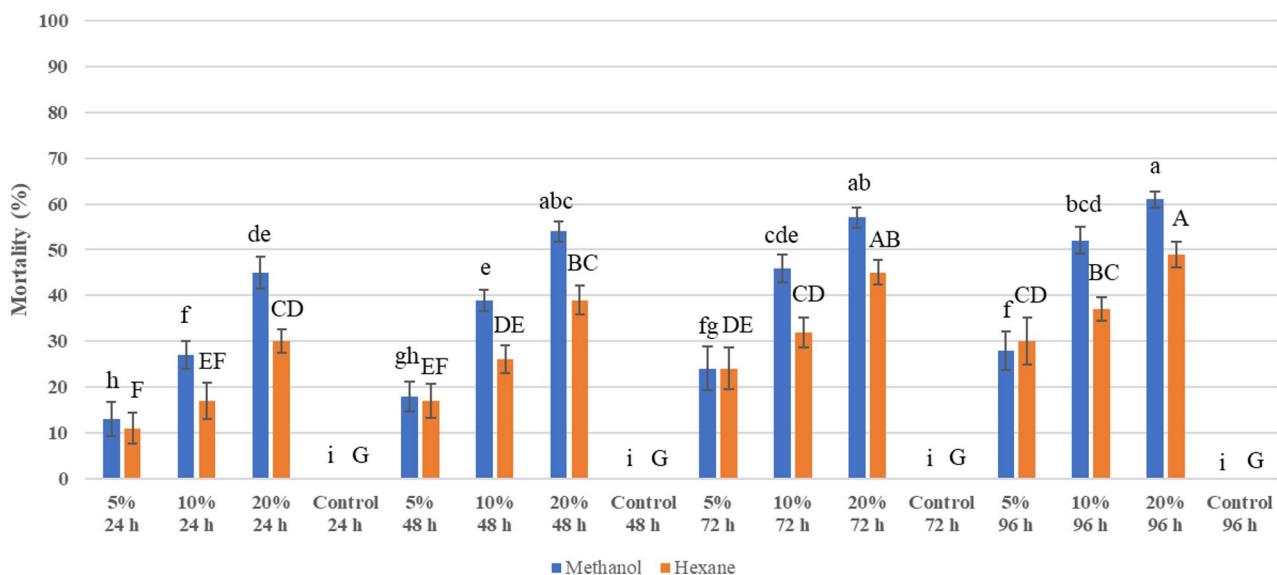
The *Gypsophila* species contains a variety of chemical compounds, including non-volatile substances such as phenolic compounds, terpenoids, saponins, cyclopeptides, and alkaloids. It also contains monoterpenes and sesquiterpenes, which are primary volatile components [8].

The primary flavones found in the aerial components of *Gypsophila* species comprise apigenin, vitexin, isovitexin, saponarin, isoorientin, orientin, and sinaroside [17–21]. Furthermore, plant triterpenoids, triterpenoid saponins, and sterols are also present [8].

Species of *Gypsophila* are rich in phenolic chemicals. Compounds such as p-coumaric acid, dihydroferulic acid, p-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, gallic acid, vanillin, and syringic acid have been found in several plant species [10, 22]. Furthermore, mangiferin, a C-glucosyl xanthone, has been detected in the buthanolic extract of the aerial parts of *G. pacifica* [18]. The content of β -sitosterol, 22,23-dihydrospinasterone, vitexin, orientin, homoorientin, hyperoside, ergost-7-en-3-ol, stigmasta-7,22-dien-3-ol (spinasterol), and stigmast-7-en-3-ol was determined by HPLC analysis of ethyl acetate extracts from the roots and aerial parts of *Gypsophila trichotoma* [23].

Saponins are richest in these species. Researchers have demonstrated the biological actions of saponins [24–26]. Numerous weeds and cultivated plants, plant diseases and plant pest have been examined in relation to saponins such β -escin, betulin, β -glycyrrhetic acid, hecogenin, oleandrin, and oleanolic acid [26–28].

Extracts against *Sitophilus granarius*

**Fig. 5** The effect of *Gypsophila pilosa* extracts on *S. granarius*. *Means in same letter were not significantly different by ANOVA ($p < 0.05$)

This study showed different efficacy levels based on the pathogen, insect, and test plants; it is the first study to examine extracts from *G. pilosa* using methanol and hexane, showing allelopathic, antifungal, and insecticide effects. There are certain sapogenin-related chemicals that have been demonstrated to have phytotoxic effects. The sterol amasterol, which was extracted from the *Amaranthus viridis*, prevented *Lactuca sativa* from germinating and growing [29]. *G. pilosa* methanol and hexane extracts were found to suppress *R. crispus*, *T. officinale*, and *T. aestivum* seed germination and seedling development when the herbicidal activity of biopesticide properties was investigated. *T. officinale* had the strongest inhibitory effect. Methanol and hexane extracts at a concentration of 3000 ppm exhibited nearly 100% suppression of germination. Other species were found to be less tolerant than *T. aestivum*.

Gypsophila species have been shown to exhibit antibacterial action in earlier research. According to a study, *Gypsophila* species were found to be effective at high concentrations against fungi that reproduce by forming mycelium, including *Alternaria solani*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. versicolour*, and *Fusarium oxysporum*. *Gypsophila pilosa* extracts in methanol, petroleum ether, and ethyl acetate were found to be efficient in inhibiting the mycelial growth of *Aspergillus niger* ATTC 10,549, *Aspergillus fumigatus* NRRL-163, and *Fusarium solani* in a different study [11]. The antifungal effects of *Gypsophila pilosa* methanol and hexane extracts on the plant pathogens *Monilia fructigena*, *Phytophthora infestans*, *S. sclerotiorum*, and *R. solani* were examined in this work. The extracts were found to be positive for *M. fructigena*, *P. infestans*, and *R. solani* mycelial growth, but not for *S. sclerotiorum* mycelial growth. The hexane and methanol extracts of *G. pilosa* exhibited significant antifungal activity, especially against *P. infestans*, *R. solani* and *M. fructigena*. *P. infestans* was the most virulent pathogen. On *S. sclerotiorum*, no effect was observed.

Research on the application of plant-based insecticides to the management of pests found in stored products has been increasing in recent years [26, 30–33]. One of the main pests, *Sitophilus* species, significantly reduces cereal yield. Paventi et al. [30] evaluated the topical contact efficacy of methanol, acetone, and n-hexane extracts of *Humulus lupulus* L. against *S. granarius*, showing that mortality rates were 100% at a dosage of 75 µg/adult in n-hexane and 85% at 37.50 µg/adult. In the acetone extract, the efficacy was 100% at a dosage of 75 µg per adult; however, it was 97.50% at a dosage of 37.50 µg per adult. In the methanol extract, the efficacy was 100% at a dosage of 75 µg per adult; however, it was 72.50% at a dosage of 37.50 µg per adult. The results suggest that methanol, acetone, and n-hexane extracts of *H. lupulus*

are efficient against *S. granarius*. Erdoğan [32] tested the damaging impact of ethanol extracts against *S. granarius* by applying extracts of *Achillea wilhelmsii* C., *Tanacetum vulgare* L., *Tanacetum parthenium* L., and *Capsicum annuum* L. at 2.5%, 5%, 7.5%, and 10% concentrations on wheat. The results showed that none of the extracts had a deadly effect. In this study, the extracts of *G. pilosa* showed deadly insecticidal activity against *S. granarius*. The mortality rate was higher with the methanol extract (up to 61%) than with the hexane extract. Both dose and application time were found to have had an effect on the insecticidal effects.

Conclusion

This study evaluated the chemical components and biological activity of *Gypsophila pilosa*, as well as its potential as a biopesticide against plant protection agents. The studies showed that the plant was rich in phenolic chemicals, particularly rutin, ferulic acid, and rosmarinic acid. Also, the high saponin concentration is thought to be one of the most important factors in maintaining the plant's biological activity. Methanol and hexane extracts were evaluated for their allelopathic, antifungal, and insecticide effects. The strong allelopathic effect on *T. officinale* and the inhibition of mycelial growth in significant fungi such as *P. infestans*, *R. solani*, and *M. fructigena*, in addition to the significant insecticidal activity against *S. granarius*, demonstrate the potential of *G. pilosa* as a potent biopesticide. However, the fact that *S. sclerotiorum* did not respond at all increases the possibility that the pathogen specific to that species' mode of action existed. Therefore, it is important to evaluate the dosage and formulation types against different plant protection agents.

Abbreviations

LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
HPLC	High-performance liquid chromatography
LOD	The limit of detection
LOQ	The limit of quantification
SPSS	Statistical Package for the Social Sciences

Supplementary Information

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Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.

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Authors' contributions

Melih Yılar, Yusuf Bayar, Hayriye Didem Sağlam Altinköy and Abdurrahman Onaran perceived the idea. Melih Yılar, Yusuf Bayar, Abdurrahman Onaran, and Hayriye Didem Sağlam Altinköy executed the experiments, collected and analyzed the data. Hayriye Didem Sağlam Altinköy wrote the initial draft of the

manuscript. Melih Yılar, Yusuf Bayar, and Abdurrahman Onaran finalized the draft and all authors approved the final version of the manuscript.

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Data availability

The publication contains the data and materials to support the study's findings.

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The present study did not include human or animal subjects. Therefore, no approval from the committee of ethics has been obtained for the present investigation.

Consent for publication

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Competing interests

The authors declare no competing interests.

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