Implications of *Euphorbia peplus* and *Euphorbia geniculata* Allelopathy on Some Plant Species and Phytopathogenic Fungi

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Invasive species of *Euphorbia peplus* and *Euphorbia geniculata* weeds compete with the crops and act as hosts for other pests, consequently interfering with the livestock. Therefore, a comprehensive allelopathic screening of *Euphorbia* spp. was implemented *via* aqueous extracts and decayed residues against *Triticum aestivum* and their associated weeds. Aqueous and ethyl acetate extracts of *E. peplus* and *E. geniculata* were suppressed by the target weeds. The effects were influenced by plant types and concentrations. The *Brassica nigra* weeds were very susceptible, while *T. aestivum* was slightly sensitive. The phytotoxicity of *Euphorbia* spp. decayed residues correlated with the used concentrations and soil properties. *Euphorbia* spp. extracts were tested against *Sclerotina sclerotiorum*, *Alternaria alternata*, and *Fusarium oxysporum* fungi. *E. peplus* at 2000 µg/mL decreased fungal growth by 57.1% (*S. sclerotiorum*), 63.1% (*A. alternata*), and 63.0% (*F. oxysporum*), while *E. geniculata* at 2000 µg/mL decreased fungal growth by 73.0% (*S. sclerotiorum*), 64.8% (*A. alternata*), and 72.7% (*F. oxysporum*). *Euphorbia* spp. allelochemicals were analysed by HPLC, which indicated the differential in secondary metabolite concentrations between the two species. These substances have a positive potential as natural pesticides that are used in the management of these species.

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INTRODUCTION

Across both tropical and temperate climates, the genus *Euphorbia* is one of the three richest genera of flowering plants. The spurge family (Euphorbiaceae) includes four giant genera, with a combined total of over 5000 species (Webster 1994; Steinmann and Porter 2002; Stejnmann and Porter 2002; Bruyns *et al.* 2006). One of the biggest and oldest plant families in the world, the Euphorbiaceae family includes about 300 genera, including approximately 8,000 species (Webster 1987). Many species of *Euphorbia* have been used in alternative and traditional medicine to treat a range of illnesses, including amaurosis, dropsy, deafness, paralysis, wounds, and skin warts (Benjamaa *et al.* 2022). The pharmacological qualities of *Euphorbia* spp. are widely utilized in medicine all over the world (Toudert *et al.* 2021). The extract (ethanolic) of *Euphorbia helioscopia* can be employed as a promising complementary and alternative therapy for diabetes mellitus

(Beltagy *et al*. 2020). Essential oils from *Euphorbia milii* produce insecticidal activity against *Periplaneta americana* and *Tettigonia viridissima*, which may exert their insecticidal efficacy through physiological disruption of ionic composition (Okonkwo and Ohaeri 2018). Many *Euphorbia* spp. have been reported to have a range of phytochemicals with widely diverse biological effects, including polyphenol compounds, flavonoids, and terpenoids (Saleh *et al.* 2019).

The genus *Euphorbia* is one of the biggest in the Egyptian flora and is found in Upper Egypt as well as the Nile Delta (Batanouny *et al.* 1991). This genus grows in different life forms, such as herbs, shrubs, and trees. It is characterized by the presence of milky latex (Boulos 1999). The genus *Euphorbia* is considered the largest one in the Egyptian flora, represented in Egypt by 42 species (El-Karemy 1994). Some species of this genus occur as noxious weeds and invasive plants in several types of cultivated areas such as rangelands, pastures, and fields and are hosts for certain pests and diseases (Tedford and Fortnum 1988; Tanveer *et al.* 2010; Pahlevani 2007). The interaction between *Euphorbia* species and crop plants is a crucial factor in agricultural productivity (Deepti *et al.* 2023). The checklist of the alien species of Euphorbiaceae in the Egyptian flora is *Euphorbia heterophylla*, *Euphorbia hyssopifolia*, *Euphorbia hirta, Euphorbia inaequilatera, Euphorbia lasiocarpa, Euphorbia mauritanica, Euphorbia prostrata, Euphorbia serpens,* and *Euphorbia nutans* (Shaltout 2016; El-Beheiry *et al.* 2020), some of which are introduced to the cultivated land as weeds and invasive plants. Commonly, invasive spurges are noxious perennials with milky white latex sap that have variable poisonous effects depending on dose, mode of exposure, and species. In pastures and rangelands, leafy spurge is a persistent, aggressive weed that easily supplants good, desirable vegetation (Messersmith 1983). *E. peplus* is initially native to Europe and North Africa (Zhi-Qin *et al.* 2010). Grazed rangelands with leafy spurge densities of 50 or above have a minimum 35% reduction in annual herbage output. Once established in pasture and rangeland environments, it tends to supplant all other plants (Lym and Kirby 1987). Many *Euphorbia*-feeding insects accept as host plants most of the species in one subgenus and reject species in the other subgenera (Pemberton 1984).

The *Euphorbia* species exhibit allelopathic activity on cereals, vegetables, forage plants, and oilseeds due to the behavior of secondary metabolites. Most *Euphorbia* species are also fungicides or natural insecticides, and they inhibit 10 to 100% of foliage production (Deepti *et al.* 2023). The invasiveness of *E. hypericifolia* could be explained by its allelopathic potential at variable concentrations on five indicator plants (Ndam *et al.* 2021). Aqueous extracts of stems and leaves of leafy spurge (*Euphorbia esula*) inhibited radicle elongation and germination of tomato and crabgrass (*Digitaria sanguinalis* L. Scop.) when 0.1 to 1.0% (w/w) of leafy spurge roots or leaves were added to the soil (Steenhagen and Zimdahl 1979). The allelopathic effect of *Euphorbia granulata* on different plant species (Hussain 1980). *E. heterophylla* has allelopathic activity of extracts from roots inhibiting 100% of germination, root, and shoot growth of the indicator *Sorghum bicolor* and *Lactuca sativa* plants (da Silva *et al.* 2019).

Allelopathic inhibitory substances included in the water extract of *E. heterophylla* are responsible for the inhibition, which harms the growth and germination of mustard (*Sinapis arvensis*), wheat (*Triticum durum*), and cucumber (*Cucumis sativus*) (Fandah *et al*. 2020). The most active constituents in several members of the genus *Euphorbia* include myricitrin, afzelin, quercitrin, rutin, quercitin, 2,4,6-tri-O-galloyl-β-D-glucose, euphorbin (A to D), kaempferol, 1,3,4,6-tetra-O-galloyl-β-D-glucose, protocatechuic acid, gallic acid, 24-methylenecycloartenol, β-sitosterol, β-amyrin, nonacosane, shikimic acid, heptacosane,

tinyatoxin, choline, camphol, rhamnose acid, and other quercitol derivatives (Kumar *et al*. 2010).

The main constraints facing crop production and its future development in newly cultivated land are weeds, especially invasive weed infestation. Through their disruption of water and mineral intake and their impact on photosynthetic partitioning, they are the cause of significant losses in agricultural yields. Invasive *Euphorbia* plants release biochemical compounds that can inhibit the growth of native plants and play crucial roles in ecosystem health and plant disease dynamics. However, there still is a need to confirm and evaluate the impact of *Euphorbia* species as the most common invasive plants in newly cultivated land on important crops. Moreover, the largest gap is the shift from evaluating their damage to crops to evaluating their impact on other weeds and their impact on phytopathogenic fungi.

Therefore, the research aimed to characterise the allelopathic potentials of two *Euphorbia* species in the environment against the nearby important plants from weeds and crops. Furthermore, a goal was to find natural products in these species that deal with the major pestilence issues that have become apparent in agricultural organisms, such as weeds and fungi. Additionally, the bioactive components were evaluated to optimise their application settings in crop productivity.

EXPERIMENTAL

Plant Materials

Euphorbia peplus and *Euphorbia geniculata* were identified by plant taxonomists in 2023 at the Desert Research Centre in Egypt after being gathered during the flowering stage from Borg El-Arab Alexandria Governorate, Egypt. Weed seeds were collected from Marryot Research Station–Desert Research Center, while the Agriculture Research Centre in Cairo provided 193 Giza wheat (seeds.

Extraction

Aqueous extraction

The dried shoot parts, weighing 25 g, were shaken on a rotary shaker for five h at room temperature after being soaked in 100 mL distilled water. Before being employed in bioassays, the mixture was filtered to remove debris and run through Whatman #4 paper, then preserved at -20 °C until use. The concentrations of 1.25, 2.5, 5, and 10 g/100 mL of the aqueous extract were achieved by diluting it with distilled water. The filter paper was placed on top of ten seeds, which were surface-sterilized in 9 cm diameter Petri dishes. Each experiment contained four replications. After that, 10 mL of extract was added, and 10 mL of distilled water was used as the control. Seven days were spent incubating petri dishes at 25 °C for 12 h of day and 12 h of darkness. The percentage of germination (G %), shoot, and root length were measured. The assays were repeated independently three times.

Organic extraction

Two hundred grams of soaking dried vegetative parts were macerated in 1000 mL of distilled water and shaken on a shaker at room temperature for five h. Following collection and filtering, the solution was successfully extracted three times with equal volume using ethyl acetate partitioning. After filtering the mixture, a rotary evaporator was used to remove the solvent. The resultant crude extracts (50 mg) were used in bioassays after being dissolved in aqueous methanol and then replaced with sterile distilled water

after the evaporation of methanol before bioassay.

Plant Bioassays

Pre-emergence activity and phytotoxicity bioassays

The ethyl acetate extract on the target plant seeds was tested at 0, 200, 400, 800, and 1200 µg/mL in DMSO. Ten seeds were put in Petri dishes with two layers of filter paper that had been wet with 10 mL of extract concentration. The control treatment was planted on filter paper that was soaked with extract-free DMSO. Germination and plant seedling length were measured following 7 days of incubation at 25 °C with a photo period of 12/12 h (dark/light).

Assays against seedlings in liquid media

After surface sterilizing by sodium hypochlorite 0.05%, *Avena fatua*, *Brassica nigra*, and *Triticum aestivum* seeds were cultivated on static Murashige and Skoog (MS) basal media for seven days or until roots and shoots appeared. The seedlings were placed in a tissue culture tube with 5 mL of liquid MS medium containing extracts at concentrations of 0, 200, 400, 800, and 1200 µg/mL. The same volume of DMSO with extract-free was used in control treatment. At 25°C, plant cultures were kept in incubators with a photo period of 12 h of light and 12 h of darkness. After 10 days of treatment, the seedlings' total biomass was measured. All experiments were designed with four replicates and repeated as needed.

Decayed Residues of *Euphorbia peplus* and *Euphorbia geniculata* in Soil

Decayed shoot materials of *Euphorbia peplus* and *Euphorbia geniculata* were tested for their phytotoxic potentials and on soil properties. The used soil was collected from Wadi Al Natroun, Egypt. Soil mechanical composition was (80.2%) sand, (14.1%) silt, and (5.6%) clay, respectively with a pH of 7.95 and electrical conductivity of 670 µS/m and contained (1.31 meq/L) sodium, (0.73 meq/L) potassium, (0.98 meq/L) calcium, and (0.62 meq/L) magnesium cations, as well as the anions (1.46 meq/L) HCO_3, (1.35 meq/L), chloride, and (1.05 meq/L) sulfate. In the greenhouse, dry plant materials were combined with soil at varying concentrations (0.0, 1.0, 2.0, 3.0, 4.0, and 5.0% (DW/100 g soil). Ten *T. aestivum* seeds were sown in the pots to test for phytotoxicity. The *T. aestivum* plant was harvested two weeks after germination, and the fresh and dry weight of the entire biomass, shoot, and root lengths, and the numbers of germinated plants were recorded. Using a Spectrophotometer, the pigments of chlorophyll A and B (Chl a and Chl b) and carotenoids were quantitatively identified. 5 g of soil from each pot were taken to determine the pH and EC values.

Bioassay against Fungi

Before usage, 50 µg of ethyl acetate crude extracts were dissolved in DMSO, and then exactly 1 mL of concentrations of 0, 250, 500, 1000, and 200 µg/mL was put into each 9 cm petri dish containing PDA media (20 mL) before it solidified. After that, the dishes were slowly turned to ensure that the crude extract was dispersed equally. One centimeter diameter (punched by sterilized Cork borer) of pathogenic *Sclerotinia sclerotiorum*, *Alternaria alternata*, and *Fusarium oxysporum* fungal growths isolated from the *Vigna unguiculata* field in Egypt was placed in the center of the dishes. After four days, data on fungal growth was collected from the dishes placed in a growth chamber with five replications at 25 °C.

Phenolic Compounds Determination

The phenolic content of the plant ethyl acetate was determined using an Agilent-1100 HPLC system with a quaternary gradient pump unit, an ultraviolet (UV) detector at 320 nm, and a C_{18} analytical column (Agilent, Santa Clara, CA, USA) measuring 150 × 406 mm with a particle size of 5 µm. Elution was done at 23 °C with a flow rate of 0.075 mL/min. The mobile phase contained 8% acetonitrile, 22% isopropyl alcohol, and 70% formic acid solution (1%). A 0.22 µm syringe filter was used to filter all dissolved standards and samples before HPLC analysis. Weed extracts were frozen for 24 h at -20 °C. The residues were then dissolved in HPLC-grade methanol and injected into the HPLC in a 20 µL volume.

Statistics

A randomized complete block design with four repetitions was used for all trials. To choose a significant difference ($P \geq 0.05$), an analysis of variance was performed using the ANOVA test. Duncan multiple ranges were then performed using IPM SPSS, 19 Software (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

The dose-response relationship of *Euphorbia* spp. shoot part aqueous extracts and the target plants exhibited that *E. peplus* achieved EC_{50} values of 8.80, 7.55, and 8.96 µg/mL in *T. aestivum* root length, shoot length, and germination, respectively. The moderate response plant was *A. fatua*, which showed values of 5.85, 4.90, and 5.65 µg/mL for root length, shoot length, and germination, respectively. However, the most vulnerable was *B. nigra*, which had EC_{50} values of 4.88, 3.95, and 6.14 µg/mL for root length, shoot length, and germination, respectively.

Similarly, the extracts of *E. geniculata* showed that *T. aestivum* was a low-sensitivity plant, as indicated from EC_{50} values of 7.99, 5.59, and 6.31 µg/mL for their root length, shoot length, and germination, respectively. The EC_{50} values in *A. fatua* were 7.99, 5.59, and 6.31 µg/mL for root length, shoot length, and germination, respectively. This was followed by *B. nigra*, the most vulnerable plant, with EC_{50} values of 3.98, 2.85, and 4.22 µg/mL for root length, shoot length, and germination respectively.

The dose-response relationship of ethyl acetate extracts revealed that the *E. peplus* EC_{50} value was 745 µg/mL in *T. aestivum* total biomass fresh weight. This was followed by *A. fatua* seedlings, for which the total biomass with EC_{50} value reached 458 µg/mL. However, *B. nigra* seedlings were the most vulnerable plant, which had EC_{50} values of 405 µg/mL in total biomass fresh weights. The maximum concentration of *E. peplus* presented reduction in the fresh total biomass by 87.1% (*T. aestivum*), 66.4% (*A. fatua*), and 82.2% (*B. nigra*), respectively.

As for *E. geniculata* shoot part extracts, the EC_{50} value was 765 µg/mL for total biomass fresh weight of *T. aestivum* seedlings. This was followed by *A. fatua* seedlings, with the total biomass coming in second and an EC_{50} value that reached 574 µg/mL. However, *B. nigra* seedlings were the most vulnerable plant with EC_{50} values of 493 µg/mL biomass fresh weight. The fresh total biomass was reduced at the highest concentration by 86.5% (*T. aestivum*), 58.0%), (*A. fatua*), and 76.3% (*B. nigra*) in that order.

Table 1. Bioassay of *Euphorbia* spp. Aqueous Extract (EC_{50}) Against Some Plant Germination and Seedling Growth in Petri-dishes

	E. peplus			*E. geniculata*		
Parameters	*T. aestivum*	*A. fatua*	*B. nigra*	*T. aestivum*	*A. fatua*	*B. nigra*
S L (cm)	8.800 ± 0.239	5.853 ± 0.740	4.880 ± 0.590	4.712 ± 1.761	7.999 ± 0.632	3.986 ± 0.342
R L (cm)	7.555 ± 0.232	4.900 ± 0.465	3.957 ± 0.335	3.491 ± 1.667	5.591 ± 0.255	2.852 ± 0.166
G%	8.963 ± 1.277	5.655 ± 1.118	6.149 ± 2.070	4.548 ± 2.236	6.312 ± 1.153	4.224 ± 1.029
F (p value)						
S L	110.711 (0.00)	114.74 (0.00)	71.54 (0.00)	267.91 (0.00)	47.17 (0.00)	63.86 (0.00)
R L	120.73 (0.00)	16.46 (0.00)	87.923 (0.00)	286.395 (0.00)	12.68 (0.01)	89.16 (0.00)
G%	95.30 (0.00)	28.31 (0.00)	92.250 (0.00)	39.300 (0.00)	120.75 (0.00)	65.93 (0.00)
SL= Shoot length, RL= Root length, G%= Germination percentage						

Table 2. Petri-dish Bioassay of *Euphorbia* spp. Ethyl Acetate Extracts Against the Tested Plants Seedling Total Biomass Fresh Weights (g)

	E. peplus			*E. geniculata*		
Conc. (µg/ mL)	*T. aestivum*	*A. fatua*	*B. nigra*	*T. aestivum*	*A. fatua*	*B. nigra*
Control	0.116	0.107	0.135	0.089	0.081	0.114
200	0.106	0.080	0.110	0.066	0.118	0.080
400	0.095	0.054	0.064	0.056	0.060	0.054
800	0.056	0.051	0.050	0.020	0.046	0.051
1000	0.020	0.042	0.032	0.015	0.033	0.042
1200	0.015	0.036	0.024	0.012	0.034	0.027
EC_{50}	744.86 ± 10.4	458.16 ± 7.9	405.23 ± 4.5	765.31 ± 6.8	573.95 ± 7.4	493.92 ± 15.7
F (*p*-value)	74.22 (0.00)	124.71 (0.00)	143.40 (0.00)	110.06 (0.00)	101.14 (0.00)	324.59 (0.00)

Table 3. Post-emergence Activity (EC_{50}) of *Euphorbia* spp. Ethyl Acetate Extracts Against Plant Seedlings

		E. peplus					*E. geniculata*				
	Conc. (µg mL)	Fresh wt. (g)	Dry wt. (g)	Chl A (mg/g)	Chl B (mg/g)	Carotene (mg/g)	Fresh wt. (g)	Dry wt. (g)	Chl A (mg/g)	Chl B (mg/g)	Carotene (mg/g)
T. aestivum	Control	3.089	0.475	4.160	4.160	2.796	3.443	0.454	4.125	4.108	2.626
	2500	0.918	0.340	2.822	2.612	2.530	0.959	0.362	2.777	2.571	2.715
	5000	0.765	0.357	2.026	1.858	1.795	0.629	0.340	2.367	1.911	1.926
A. fatua	Control	2.668	0.424	3.432	3.637	2.507	2.974	0.377	3.362	3.565	2.763
	2500	1.416	0.330	2.757	2.584	2.499	1.578	0.294	2.713	2.544	2.681
	5000	1.189	0.250	1.971	1.834	1.769	1.015	0.222	1.940	1.805	1.898
B. nigra	Control	3.293	0.489	3.360	3.580	2.728	3.671	0.482	3.291	3.508	2.548
	2500	2.826	0.374	2.777	2.593	2.509	2.587	0.333	2.733	2.552	2.692
	5000	1.120	0.326	1.988	1.841	1.777	1.249	0.325	1.956	1.812	1.907
F (*p* value)											
	Species	1184.2 (0.00)	66.4 (0.00)	101.8 (0.00)	31.4 (0.00)	30.1 (0.00)	287.2 (0.00)	72.2 (0.00)	110.7 (0.00)	34.2 (0.00)	32.7 (0.00)
	Conc.	617.8 (0.00)	18.9 (0.00)	4701.3 (0.00)	4169.6 (0.00)	2120.9 (0.00)	808.9 (0.00)	1119.4 (0.00)	2350.6 (0.00)	2084.8 (0.00)	1060.4 (0.00)
	Species * Conc.	313.4 (0.00)	11.6 (0.00)	31.3 (0.00)	4.1 (0.02)	42.1 (0.00)	340.7 (0.00)	12.6 (0.01)	34.0 (0.00)	11.40 (0.01)	45.80 (0.00)

Under the greenhouse conditions, the post-emergence activities of *E. peplus* and *E. geniculata* ethyl acetate extracts at 2500 and 5000 µg/mL were evaluated on *T. aestivum* and associated weeds of *A. fatua*, and *B. nigra* at 4-leaf stage seedlings (Table 3). Concerning to application of spraying *E. peplus* extracts at the maximum concentration, fresh weights, dry weights, carotenes, and Chl A and Chl B were reduced by 75.2, 24.8, 51.3, 55.3, and 35.8% (*T. aestivum*), 55.4, 41.0, 42.6, 49.6, 49.6, and 29.4% (*A. fatua*), and 66.0, 33.3, 40.8, 48.6, and 34.9% (*B. nigra*), respectively, in comparison to the untreated control. As for *E. geniculata* extracts at the maximum concentration, it resulted in reduction in fresh weights, dry weights, and carotenes of 81.7, 25.1, 42.6, 53.5, and 26.6% (*T. aestivum*), 65.9, 41.1, 42.3, 49.4, and 31.3% (*A. fatua*), and 66.0, 32.6, 40.6, 48.3, and 25.2% (*B. nigra*) in comparison to the control. Statically, there were significant interactions between species × concentration in fresh weights (F=313.4, $p \leq 0.00$), fresh weights (F=11.60, $p \leq 0.00$), carotenoids (F=31.3, $p \leq 0.00$), Chl A (F=4.1, $p \leq 0.000$), and Chl B (F=42.1, $p \leq 0.00$) respectively for *E. peplus*. Meanwhile, there was a significant interaction effect in fresh weight (F=340.7, $p \leq 0.000$), dry weight (F=12.60, $p \leq 0.00$), carotenoids (F=34.00, $p \leq 0.000$) Chl A, (F=11.40, $p \leq 0.00$) Chl B, and (F=45.80, $p \leq 0.00$) respectively for *E. geniculata*.

In soil, decayed residues of *Euphorbia* species were tested using *T. aestivum* seeds at concentrations of 2, 3, 4, and 5% (W/W) for two weeks after germination (Table 4). The concentration of 1% stimulated growth, but other concentrations reduced *T. aestivum* growth parameters. The greater reduction reached its peak at 5% concentration of *E. peplus* decayed residues, in shoot length, root length, fresh weight, dry weight, Chl A, Chl B, carotenes by 21.3%, 17.1%, 27.9%, 24.9%, 11.8%, 24.8 and 21.5% respectively. At the highest concentration (5%) of *E. geniculata* decayed residues, reduction was achieved in shoot length, root length, fresh weight, dry weight, Chl A, Chl B, carotenes by 12.1%, 20.2%, 25.0%, 20.0%, 6.0%, 3.6%, and 16.9%, respectively. As for soil parameters, the decayed residues of *E. peplus and E. geniculata* caused a reduction in soil pH; however, a little increase was detected in soil EC values, which reached 2.8 and 2.7%, respectively. These results pointed out that *E. peplus* had a greater impact on soil and *T. aestivum* seedling characteristics than *E. geniculata*. Statically, there were significant interactions between species × concentration in shoot length (F=3.66.4, $p \leq 0.037$), root length (F=12.92, $p \leq 0.00$), fresh weights (F=2.99, $p \leq 0.035$), dry weights (F=3.02, $p \leq 0.025$), carotenoids (F=4.09, $p \leq 0.029$), Chl A (F=4.12, $p \leq 0.025$), and Chl B (F=12.1, $p \leq 0.00$), respectively.

Antifungal Activity of *E. peplus* and *E. geniculata* Ethyl Acetate Extracts against Some Plant Pathogenic Fungi

The ethyl acetate extracts of *E. peplus* and *E. geniculata* impact on the growth of *S. sclerotiorum*, *A. alternata*, and *F. oxysporum* were demonstrated in Table 5. *E. peplus* extracts were tested against the growth at concentrations of 250, 500, 1000, and 2000 µg mL. They dramatically decreased fungal growth by 4.8, 21.4, 40.5, and 57.2%, (*S. sclerotiorum*) 19.0, 23.8, 33.3, and 63.1% (*A. alternata*), 13.6, 41.0, 58.9, and 63.0%, (*F. oxysporum*), respectively, in comparison to its control. Meanwhile, when *E. geniculata* extracts were tested against the growth at concentrations of 250, 500, 1000, and 2000 µg mL, they dramatically decreased fungal growth reaching 19.6, 56.8, 59.2, and 73.0% (*S. sclerotiorum*), respectively, 22.0, 39.9, 61.3, and 64.8% (*A. alternata*), and 14.8, 38.1, 59.5, and 72.7%, (*F. oxysporum*) respectively, compared to the control.

Table 4. Effect of *Euphorbia* spp. Ethyl Decayed Residues on Wheat Seedling Growth and Soil Parameters

		T. aestivum							Soil	
	Conc. (% w/w)	SL (cm)	RL (cm)	Fresh wt. (g)	Dry wt. (g)	Chl A (mg/g)	Chl B (mg/g)	Carotene (mg/g)	pH	EC (µs/cm)
E. peplus	Control	23.500	17.500	12.500	4.010	2.550	2.220	1.490	7.900	670.010
	1%	23.500	17.440	12.500	3.920	2.560	2.250	1.530	7.900	670.000
	2%	21.000	15.780	10.230	3.410	2.280	1.950	1.380	7.900	680.900
	3%	20.500	14.840	10.140	3.380	2.280	1.790	1.240	7.800	681.580
	4%	19.000	14.760	9.050	3.020	2.280	1.790	1.190	7.700	683.600
	5%	18.500	14.500	9.010	3.010	2.250	1.670	1.170	7.600	688.500
E. geniculata	Control	24.000	17.550	13.130	4.100	2.690	2.250	1.480	7.900	669.810
	1%	24.500	17.520	13.740	4.180	2.750	2.270	1.490	7.900	669.810
	2%	22.000	15.770	10.430	3.480	2.690	2.230	1.450	7.900	677.470
	3%	22.000	15.270	10.030	3.340	2.620	2.060	1.280	7.800	676.130
	4%	21.500	14.510	9.880	3.290	2.620	2.170	1.350	7.700	682.110
	5%	21.090	14.010	9.850	3.280	2.530	2.170	1.230	7.700	687.900
F (*p* value)	Conc.	152.50 (0.00)	154.70 (0.00)	137.26 (0.00)	44.62 (0.00)	37.93 (0.00)	38.70 (0.00)	360.33 (0.00)	8.5 (0.00)	15.59 (0.00)
	Species	149.26 (0.00)	120.35 (0.00)	120.13 (0.00)	30.96 (0.00)	27.50 (0.00)	34.30 (0.00)	299.38 (0.00)	5.6 (0.00)	12.12 (0.00)
	Conc. x Species	3.66 (0.037)	12.92 (0.00)	2.99 (0.035)	3.02 (0.025)	4.09 (0.029	4.12 (0.025)	12.21 (0.00)	0.67 (0.65)	1.08 (0.99)
SL= Shoot length, RL=Root length										

Table 5. Activity of *Euphorbia* spp. Ethyl Acetate Extract Against the Tested Fungi Growth (cm)

	E. peplus			*E. geniculata*		
Conc. (µg/ml)	*S. sclerotiorum*	*A. alternata*	*F. oxysporum*	*S. sclerotiorum*	*A. alternata*	*F. oxysporum*
Control	8.400	8.400	7.040	8.400	8.400	6.899
250	8.000	6.800	6.080	6.750	6.552	5.874
500	6.600	6.400	4.153	3.626	5.050	4.273
1000	5.000	5.600	2.895	3.426	3.248	2.791
2000	3.600	3.100	2.601	2.264	2.958	1.881
EC_{50}	613.22 ± 13.5	1215.45 ± 14.2	403.31 ± 18.4	420.627 ± 11.3	513.73 ± 9.8	571.03 ± 9.8
F (*p-value*)	91.643 (0.00)	43.930 (0.00)	568.333 (0.00)	21.187 (0.00)	49.279 (0.00)	445.550 (0.00)

Table 6. *Euphorbia* spp. Ethyl Acetate Extracts Quantitative Determination Using HPLC-UV

	Contents (μg g^{-1})	Rt (min)	*E. peplus*	*E. geniculata*
1	Hydroxycinnamic acid	27.97	25.600 ± 0.25	26.120 ± 0.30
2	P-Hydroxybenzoic acid	9.26	23.840 ± 0.20	24.360 ± 0.21
3	Syringic acid	11.63	32.840 ± 0.19	33.510 ± 0.22
4	Gallic acid	12.27	33.650 ± 0.21	34.340 ± 0.26
5	Chlorogenic acid	13.80	31.900 ± 0.16	30.310 ± 0.21
6	Vanillic acid	15.65	19.250 ± 0.12	18.600 ± 0.16
7	Caffeic acid	17.52	38.030 ± 0.27	38.810 ± 0.23
8	Coumaric acid	19.25	47.030 ± 0.19	47.990 ± 0.17
9	Kaempferol	20.52	37.070 ± 0.18	38.370 ± 0.31
10	Ferulic acid	22.31	25.530 ± 0.31	26.060 ± 0.14
11	Citric acid	25.40	38.400 ± 0.23	37.700 ± 0.27
12	Salicylic acid	29.12	22.030 ± 0.20	22.480 ± 0.11
	Total concentration		371.18	378.65

According to analysis by HPLC-UV, the ethyl acetate extracts of *Euphorbia* species that were qualitatively present contained twelve free chemicals based on their relative retention times that were identified as hydroxycinnamic acid, *p*-hydroxybenzoic acid, syringic acid, gallic acid, chlorogenic acid, vanillic acid, caffeic acid, coumaric acid, kaempferol, ferulic acid, citric acid, and salicylic acid substances. The quantitative analysis revealed that the amounts of coumaric acid in *E. peplus* and *E. geniculata* reached approximately 47.0 and 48.0 µg g^{-1} DW, respectively. However, small amounts of vanillic acid 19.2 and 18.6% µg g^{-1} DW were found in *E. peplus* and *E. geniculata*, respectively. The results showed these weeds are abundant in physiologically active substances (Table 6).

DISCUSSION

Euphorbia species are important to agriculture because they compete with crops for nutrients, CO_2, and water resources and serve as hosts for several pests. However, few studies on the allelopathic effects of *E. peplus* and *E. geniculata* have been conducted. Therefore, the allelopathic effects of *E. peplus* and *E. geniculata* were assayed against wheat crop (*T. aestivum*) and associated weeds (*A. fatua* and *B. nigra) via* aqueous leachate and on their soil chemistry *via* decayed residues. Additionally, the antifungal effect of these species was tested on *S. sclerotiorum*, *A. alternata*, and *F. oxysporum*. In the Euphorbiaceae family, great phytochemical varieties were found, including tannins (Giordani *et al.* 2001), terpenoids (Liu *et al.* 2002), and phenolics (Duarte *et al.* 2008). Genus *Euphorbia* members cover macrocyclic diterpenoid compounds that have analgesic, antimicrobial, anticancer, PGE2-inhibitory, and anti-HIV properties (Jassbi 2006). However, the *Euphorbia hypericifolia* plant showed that certain allelochemicals might be used to create natural compounds that promote plant growth and can served as bioherbicides (Ndam *et al.* 2021).

The dose-response relationship of *Euphorbia* species extracts offered important information about their allelopathic capabilities and specificity. It revealed that *E. peplus* and *E. geniculata* aqueous extracts have varying phytotoxic effects on test plant species, based on the concentration used in the current study. This aligns with Ndam *et al.* (2021), whose study found that variable allelopathic patterns depend on *E. hypericifolia* concentration against five indicator plants; this may be attributed to the increase of active constituents. The lowest sensitivity shown by the *T. aestivum* plant could be because of their variation in the allelopathic substances absorption, translocation, and physiological or biochemical response of the target species that detoxify the allelopathic molecules. Conversely, weeds' high susceptibility could be caused by increased absorption, ineffective detoxification, or increased sensitivity to particular extract constituents, to which *B. nigra* was more sensitive than *A. fatua* to *Euphorbia* species allelochemicals. Similar patterns were shown in ethyl acetate extracts of *E. peplus* and *E. geniculata*, whereas more powerful activities were displayed from the extraction of ethyl acetate with a stronger impact as compared to water extracts. The root, leaf, stem, and fruit of *E. helioscopia* watery extract decreased the germination index and seed germination (lentils and chickpeas), while the leaf extract lengthened the average germination time for all tested plant crops (Tanveer *et al.* 2010). The extract of *E. dracunculoides* showed a significant reduction in the plumule and radicle length of Chickpea (Kil and Yun 1992). The stimulatory and inhibitory effects of *Euphorbia hirta* on germination rate and seedling

growth in response to particular doses demonstrate its allelopathic action on sorghum (Mali *et al.* 2021).

The decomposed residues of *Euphorbia* species in the soil emphasized the unique impacts of their allelopathic abilities against other plants and soil properties. The differences between *E. peplus* and *E. geniculata* were found in *E. peplus'* decayed process compared to remarkable inhibitory effects during the decayed process than *E. geniculata* against *T. aestivum* seedling development and soil characteristics. These impacts of *Euphorbia species* could serve for a better understanding of invasion and impacts in both agriculture and soil systems. The decayed effect of *E. peplus* was higher than *E. geniculata*. These findings are proven by Singh *et al.* (2003), who informed the reduction in the dry weight of wheat grown in soil infested with *E. helioscopia*. Abu-Romman *et al.* (2010) found that *Euphorbia hierosolymitana* exhibited an allelopathic effect on wheat seedling growth, seed germination, chlorophyll, and protein. According to Choudhary *et al.* (2023), researchers divided the allelochemical mode of action into direct and indirect categories, including functional and genuine (true) allelopathy. Allelochemicals have two possible effects: direct allelopathy, which affects the target directly, and secondary degradation products, which are discharged into the soil and can either damage plant development or alter the microenvironment, which affects growth indirectly. Moreover, the allelochemicals released from weeds, most of them water-soluble chemicals, into the soil have allelopathic effects on wheat seed germination and growth (Al-Qthanin *et al.* 2024).

The plant pathogen of *S. sclerotiorum*, *A. alternata,* and *F. oxysporum* fungi are among the most dangerous diseases in many crops. They lead to huge decreases in the growth parameters and losses in crop yield. Therefore, the current study investigated the antifungal potential of *E. peplus* and *E. geniculata* ethyl acetate extracts under laboratory conditions with a series of concentrations. The results showed that *E. peplus* and *E. geniculata* ethyl acetate extracts have antifungal activity against *S. sclerotiorum*, *A. alternata,* and *F. oxysporum* significantly by reducing fungal growth compared with the control. *E. peplus* extract has significantly inhibited the growth of *S. sclerotiorum* and *F. oxysporum*, while extracts of *E. geniculata* show broad-spectrum action and successfully prevented the growth of *F. oxysporum*, *A. alternata*, and *S. sclerotiorum*. Differences in the fungi's susceptibility based on the bioactive chemicals in the extracts of *E. peplus* and *E. geniculata's* vary in efficacy against particular fungal species. Chemical analysis points to their capacity to prevent the growth of fungi, particularly when combined. However, the potential of *Euphorbia* species as a source of naturally occurring antifungal chemicals is supported by earlier research that found *Euphorbia* species hydroalcoholic extract of this species showed antimicrobial activity against pathogenic bacteria, including Gram-negative (*K. pneumonia, S. typhi, P. aeruginosa,* and *E. coli*) *and* Gram-positive bacteria such as *Staphylococcus aureus* and *Candida albicans* yeast (Bahy *et al.* 2022). *Euphorbia* spp. active metabolites, including lathyrane diterpenoids, especially *Euphorbia* factor L3 have excellent inhibitory effects against *Phytophthora capsica* plant pathogen. Plant-derived terpenoids' antifungal properties have been partially ascribed to their damaging effects on the cell membrane as well as the cell wall of pathogens (Wang *et al.* 2023). Moreover, *Euphorbia* factor L3 compound has anticancer activity and induces apoptosis by loss of mitochondrial pathway and release of cytochrome *c* (Zhang *et al.* 2011).

The phytochemical screening of *E. peplus* and *E. geniculata* extracts revealed the presence of phenolic and flavonoids. The biological activity against plants and fungi is highlighted by the varying amounts of secondary constituents in both *E. peplus* and *E. geniculata*. The *E. peplus* had higher quantities of flavonoids and phenolic acids, which

makes it a more potent allelopathic competitor to *E. geniculata*. However, *E. geniculata* is a more potent antifungal agent than *E. peplus* due to its higher concentrations of flavonoids and phenolic acids. Strong antifungal and allelopathic effects are well-known for phenolic acids, such as coumaric, ferulic, and caffeic acids. Since caffeic and ferulic acids can break down fungal cell walls and prevent spore germination (Macías *et al.* 2007; Simonetti *et al.* 2020). Phenolic compounds and their derivatives have been frequently cited as chemical sources of allelopathy (Rice 1984). *E. peplus* displayed higher amounts of chlorogenic acid, hydroxycinnamic acid, and citric acid, which may account for its superior allelopathic effectiveness. On the other hand, *E. geniculata* has greater concentrations of other chemicals, which are mostly linked to antioxidant properties and may not be as efficient against fungus. These variations imply that *E. geniculata* and *E. peplus* had higher concentrations of non-specific phenolic and a flavonoid that is responsible for allelopathic and antifungal properties. Whereas, the presence of citric acid may help to modify the pH of the environment, which may not directly have an antifungal effect but may indirectly affect microbial activity. Salicylic acid, coumaric acid, and hydroxycinnamic acid are essential for allelopathy. *E. peplus* may have a competitive advantage because of its greater levels of chlorogenic acid, hydroxycinnamic acid, and citric acid, which may prevent nearby plants from germinating and growing. Since *E. geniculata* has fewer of these allelopathic substances, it probably has less of the inhibiting chemicals. Possible synergistic or antagonistic interactions between the chemicals may be bioactivity constituents and between the target plants. This result validates previous findings by Ndam *et al.* (2014), who found that the presence of phenolics in the leaf extracts of *E. hypericifolia* could thus have significantly advanced the suppression of germination, growth, and biomass production in this study's targeted plants. Katemas (*Euphorbia geniculata* Ortega) toxicity exhibited the highest mortality at 5% concentration in armyworm larvae (*Spodoptera litura*). While spectroscopy analysis identified a type of pentacyclic triterpenoid compound, namely lupeol acetate (Eliza *et al.* 2016). In addition, non-polar secondary metabolites of *Euphorbia peplus* may serve as potential therapeutic candidates for leishmaniasis (Amin *et al.* 2017).

CONCLUSIONS

1. The results of this work highlight the significant effectiveness of *Euphorbia* species on plants and fungi and the great convergence between the two laboratory plants (*T. aestivum, A. fatua,* and *B. nigra*). These results may be due to the closest relationship of the genetic diversity observed between *E. geniculata* and *E. aphylla*; and *E. pulcherrima* and *E. peplus* using RAPD-PCR.
2. These findings demonstrated that *E. peplus* and *E. geniculata* extracts were effective due to their allelochemicals, which give them their allelopathic capabilities. These potentials are concentration-dependent and demonstrate stimulatory and phytotoxic effects on germination and growth traits in the sensitivity order of *B. nigra* > *A. fatua* > *T. aestivum* of targeted plants.
3. The findings demonstrate the potential of the bioactive chemicals in *Euphorbia* species as natural substitute antifungal agents for the control of *F. oxysporum* > *S. sclerotiorum* > *A. alternata* of target fungi. Therefore, these plants are a source of extracts with suppressive properties as potential natural pesticides.

Finally, the allelopathic effects of two invasive *Euphorbia* species, *Euphorbia peplus* and *Euphorbia geniculata*, significantly impact other weed species and phytopathogenic fungi. This dual action not only holds promise for competing with other weed plant species and control of some phytopathogenic fungi as potential natural pesticides, but it also may have promising usage in other agriculture. These results highlight the necessity of taking action to manage these invasive weeds to achieve sustainable agriculture and establish a baseline for economic crop productivity away from the use of chemically manufactured pesticides.

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Author Contributions

Conceptualization, Investigation, Methodology (A.A.E. Ateya), Analysis, investigation, methodology, writing ± original draft (M. A. El-Sakhawy), Data analysis, Methodology, writing and review (M. Balah).

Conflict of Interest

The authors confirm that this article's content has no conflict of interest.

Data Availability Statement

All data generated or analyzed during this study are available from the corresponding author upon justified request.

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