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ORIGINAL ARTICLE

Allelopathy of invasive weed *Solanum elaeagnifolium* Cav.: an investigation in germination, growth and soil properties

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Abstract

Solanum elaeagnifolium Cav. is known to be one of the most invasive species worldwide. In this study, laboratory and greenhouse experiments were carried out to investigate the allelopathic properties of *S. elaeagnifolium* vegetative parts, root parts, fruit mucilage, and exudate extracts on plant communities and soil properties. In addition, the extract profiles of allelochemicals were quantified and their influence on soil properties and microorganisms was determined. Overall, the allelopathic performance of *S. elaeagnifolium* was established depending on the extract types, used concentrations, and target species. The dose-response activity indicated that vegetative parts extract showed the greatest allelopathic potential followed by root parts extract. Subsequently, mucilage extract had a moderate inhibitory potential, while root exudates showed the least activity. The same trend with slight response was detected in soil properties of pH and EC properties. Polyphenols, in the range of 5.70–0.211 mg · g^{-1} and flavonols, in the range of 2.392–0.00 mg · g^{-1}, were found in the analyzed samples extracted by ethyl acetate using LC-DAD-MS. The total phenol amount was 1.67 to 1.89 in the rhizosphere and 0.53 to 087 mg · g^{-1} in non-rhizosphere soils. *Solanum elaeagnifolium* exhibited a greater significant suppression of fungi count in both high and low-density areas than in rhizosphere bacteria. In conclusion, the strong and broad-spectrum allelopathic potentials may enhance the ability of *S. elaeagnifolium* to impact seed germination and seedling growth of neighboring species. These biochemical weapons may play a critical role to facilitate their invasion and establishment in new agroecosystems.

Keywords: allelopathy, allelochemicals, invasive weeds, soil properties, weed management

Introduction

Invasive alien species (IAS) pose threats to human health, food supplies, and national security (Yan *et al.* 2017). Potentially they can displace native plants and crop species due to their allelopathy properties and competition for space, nutrients, water, and light (Wardle *et al.* 1994; Mack and D'Antonio 1998). They have a multitude of impacts on plant communities through their effects on soil chemistry and ecosystem function (Weidenhamer and Callaway 2010). Understanding the biology of an invasive plant species could help to successfully facilitate their management (Chauhan and Johnson 2010). Measures toward preventing biological invasions are needed to prevent adverse impacts from future invasions (Weber and Li 2008). *Solanum elaeagnifolium* Cav. (Solanaceae) is one of the worst invasive alien plants worldwide (Brunel 2011), invading all Mediterranean Basin countries (Mekki 2007), including Egypt (Täckholm 1974;

Balah 2011). It is a noxious invasive weed in dry areas worldwide (Knapp *et al.* 2017). *Solanum elaeagnifolium* infestation has caused high economic losses in cotton, grain sorghum, wheat, and lucerne (Boyd and Murray 1982; Lemerle and Leys 1991). It has also interfered with peanut growth (Hackett *et al.* 1987), resulting in up to 75% yield loss. Furthermore, it has an indirect effect by harboring pests and diseases of plants (Uludag *et al.* 2016). Allelopathy is defined as any direct or indirect harmful interaction between plants (including microorganisms) through chemicals released into the environment (Rice 1974). It is well known as one of the plant invasion mechanisms with chemical, physiological and ecological implications (Callaway and Aschehoug 2000; Callaway *et al.* 2008; Thorpe *et al.* 2009) and helps in our understanding of successful invasion (Uddin *et al.* 2017). Whereas the invasion may be successful due to allelochemicals (Callaway and Ridenour 2004), these allelochemicals are highly inhibitory to the resident plants in the introduced range (Hierro *et al.* 2005). Invasive plants have strong allelopathic effects (Hu and Kong 1997; Inderjit *et al.* 2008), because of the allelochemicals that are released by leaching, root exudation, volatilization, residue decomposition, and other processes in agricultural systems (Chou 1990). Successful invasions of plants are based on allelopathic interactions between the introduced plants and native plant communities (Ning *et al.* 2016). The exotic plants exude phytotoxic compounds that are novel in invaded areas (Jandova *et al.* 2015), and affect the native plants (Parepa and Bossdorf 2016). *Solanum elaeagnifolium* is a common agronomic weed that competes with crops, exudes plant inhibitors, interferes with animal husbandry and harvesting practices, and serves as an alternate host for phytophagous insects and plant diseases (Boyd *et al.* 1984).

Numerous Solanaceae have a great variety of steroidal saponins which are of interest to both environmental and human health (Keeler *et al.* 1990; Zygadlo 1994; Colmenares *et al.* 2010). Glycoalkaloids (Silva *et al.* 2005) are of interest due to their structural diversity and significant biological activities (Sparg *et al.* 2004). *Solanum elaeagnifolium* has anti-inflammatory, analgesic, antioxidant and hepatoprotective activities (Badawy *et al.* 2013). It is a potential source of antibacterial agents for antibiotic-resistant bacteria (Amer *et al.* 2013). It exhibits antioxidant effects and can potentially alleviate diabetic complications (Houda *et al.* 2014). *Solanum elaeagnifolium* contains solamargin, solasonine, α-, β-solanine (Delabays *et al.* 2004), and tropane alkaloids solanine (Buck *et al.* 1960). Kaempferol and kaempferol 3-glucoside as monoacylated flavonoid glucosides can be isolated from its aerial parts (Chiale *et al.* 1991). Glycoalkaloids are isolated from their seeds and leaves as bioactive molluscicidal compounds (Bekkouche *et al.* 2000). The phytotoxic effect of *Solanum* sp. is related to some glycoalkaloid derivatives (Grazi and Myers 1990; Sun *et al.* 2010). The most active isolates in seeds of *S. elaeagnifolium* are chlorogenic acid kaempferol 3β-D-(6-O-cis-cinnamoyl glucoside) which decreased the total biomass fresh weight of *P. oleracea* (Balah 2015). The foliage water-soluble extracts of *S. elaeagnifolium* inhibited germination and root growth of cotton and lettuce, respectively (Bothma 2002). Hydroxyl-3-methoxyflavone, quercetin, kaempferol 3β-D-(6-O-cis-cinnamoyl glucoside) and chlorogenic acid are phytotoxic constituents isolated from silver nightshade leaves on *Convolvulus arvensis* weeds (Balah and Abdelrazik 2020). We hypothesized that the invasive weeds have strong and broad-spectrum allelopathic capabilities against resident plants. Thus, *S. elaeagnifolium* was chosen to investigate the allelopathic potential on the associated species to confirm their harmful ecological impacts in invaded sites. This study was conducted in an attempt to identify the allelopathic properties of invasive *S. elaeagnifolium* on the number of weeds and crops and its effect within the rhizosphere on microbes and soil properties. This type of knowledge can be useful in developing new environmental risk assessments to prevent the spread of invasive weeds and can be used as a potential method for weed control.

Materials and Methods

Plant materials

Samples of *S. elaeagnifolium* individuals were collected from Borg El-arab and El-Hammam regions of Egypt during 2019/2020 randomly at 30 52 260°N, 029 29 008°E and 30 51 057°N, 029 26 280°E. These areas have a moderate climate where the average temperatures are between 18 and 30°C. It receives more than 50 mm of rain in winter and the average relative humidity is approximately 65% per year. The vegetative parts were obtained by cutting with a manual cutter during the flowering stage. Seeds and roots were collected by hand at the harvest stage after plowing. The plant sections included shoots (vegetative), roots, and seeds which were separated to dry, then, milled, and sieved through a 20–40 mesh. The powder was held in paper bags under laboratory conditions before being used at the Desert Research Center, Mataria, Cairo, Egypt. The tested plants included the crops of wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L. subsp. *vulgare*), faba bean (*Vicia faba* L.), onion (*Allium cepa* L.), Egyptian clover (*Trifolium alexandrinum* L.), alfalfa (*Medicago sativa* L.), maize (*Zea mays* L.) and the weeds of bindweed (*Convolvulus arvensis* L.), canary grass (*Phalaris minor* Retz.), wild oat (*Avena*

fatua L.), rabbit foot grass [*Polypogon monospeliensis* (L.) Desf.], jute mallow (*Corchorus olitorius* L.), and purslane (*Portulaca oleracea* L.).

Preparation of the aqueous extracts of *Solanum elaeagnifolium*

Two hundred powders of each of the vegetative and the subterranean parts (root) were put into 2,000 ml deionized water in glass bottles and placed on a rotary shaker at 160 RPM for 12 h at lab temperatures. The mixture was collected to remove fiber and centrifuged at 4,000 RPM for 15 min, then the filtrated through a sterile syringe filter 45 µm, followed by 22 µm pores to be sterilized before being stored at –20°C until used. The stock concentration of the extract was 10% (10 g dry weight · 100 ml^{-1} water) diluted to produce six concentrations (0, 1, 2, 4, 6, 8 g · 100 ml^{-1}) before treatments.

Preparation of mucilage extracts of *Solanum elaeagnifolium*

Mucilage (locular tissue) was extracted from fruits manually after removing the seeds by adding distilled water (1 liter) and shaking overnight at room temperatures. This process was repeated several times to remove all mucilage from fruits and seeds. The obtained supernatants were extracted by partitioning with an equal volume of ethyl acetate solvent. The filtrates had been dried with a rotary evaporator under a vacuum at 40°C. The residues were weighed and kept at –20°C until used. The assays were conducted by diluting the mucilage residues to: 0, 125, 250, 500, 750 and 1,000 mg · 100 ml^{-1} in 50% aqueous methanol. Then the methanol was evaporated and replaced by distilled water before treating the target seeds.

Preparation of *Solanum elaeagnifolium* root exudates

Sterilized *S. elaeagnifolium* seeds were placed on static Murashige and Skoog (MS) basal media after soaking for 24 h in 1 g · l^{-1} gibberelic acid (GA), then, incubated in 12/12 dark/light at 15/25°C and 80% humidity for 7 days. The germinated seedlings were transferred to tissue culture tubes containing 10 ml of MS liquid media. After 28 days in a rotary shaker in a culture room, exudates of water were collected and subject to refrigeration (–20°C) and lyophilized by freeze-drying to dryness. The residues were suspended in 50 ml distilled water with pH ≤ 4, followed by a liquid-liquid partitioning step and phase separation by adding ethyl acetate three times (extract suspension in water and ethyl acetate). The filtrates were vacuum-dried with a rotary evaporator. These extracts were re-suspended in 50% methanol and diluted to 0, 125, 250, 500, 750, 1,000 and 1,500 µg · ml^{-1} and tested for 10 days against tested plants.

Comparison of shoot, root, mucilage and exudates extracted by ethyl acetate

Aqueous extracts of the shoot and root parts, mucilage water, as well as root exudates of *S. elaeagnifolium* were extracted by partitioning with ethyl acetate with equal volumes after adjustment of the water leachates to pH = 4. The ethyl acetate extract was dried with a rotary evaporator under a vacuum at 40°C. The residues were weighed and the mucilage extract residues were diluted to 0, 12.5, 25, 50, 100 and 200 µg · ml^{-1} in distilled water on the previous plants. Seeds of *T. aestivum, T. alexandrinum, M. sativa, Z. mays* crops as well as *C. arvensis, P. oleracea*, and *C. olitorius* weeds were grown for 3–4 days. Then one seedling was transferred to each tissue culture tube, with 5 ml of MS media plus the extracts and 15 days later, seedlings were removed and washed by dry weight (DW) and total biomass fresh weights were recorded.

Laboratory bioassay

To investigate the allelopathic activity, 10 sterilized seeds were added onto filter paper in a Petri dish (9 cm diameter) with 10 ml of *S. elaeagnifolium* extracts of different concentrations. Petri dishes were kept in incubators for seed germination at 25 ± 2°C, 12 h light per day, and 80% humidity. Each procedure was repeated at least twice with four replicates and placed randomly in incubators for 7 days. Seed germination percentages and growth of seedlings (radical and hypocotyl length) were calculated. EC_{50} values were calculated by plotting concentration on a log scale (X) and the reduction response on the Y axis. The data appear linear which is the point signed in a semi-log graph paper.

Greenhouse bioassay

A pot experiment was performed to test shoot and root parts, mucilage water, and root exudates of *S. elaeagnifolium* under greenhouse conditions. These pots were filled with 500 g sand and peat moss (1 : 1) and sowed with 10 seeds of *T. aestivum* with five replications. The pots were treated with 250 ml of aqueous extracts (alternative to irrigation) divided into five times of 50 ml at 0, 2, 4, 8, and 12 days. The tested concentrations were 0, 25, 50, 100 and 200 µg · ml^{-1} of ethyl acetate extracts, whereas distilled water was used in the control treatment. Two weeks after germination started seedling lengths and the number of germinated seeds were calculated to determine the seedling vigor

index (SVI) = germination % × (Root length + Shoot length), while soil pH and EC were determined according to Inderjit (2002) at the end of the experiment.

6-Quali-quantitative determination of water-soluble allelochemicals

Phenol analysis was conducted by extracting the aqueous extractions with ethyl acetate at room temperature. The extracts were filtered, evaporated to dryness, and re-dissolved in 1 ml MeOH before the analysis. Phenolic compounds were analyzed by LC-DAD electrospray ionization (ESI)-MS analysis. Analyses of flavonols and phenolic acids were carried out using a liquid chromatograph equipped with a DAD detector (Waters Corporation, Milford MA 01757, USA). Compounds were separated using a 150 × 4.6 mm C_{18} column. UV/Vis spectra were recorded in the 190–600 nm range and the chromatograms were acquired at 220, 240, 280, 330 and 350 nm. The samples were analyzed by gradient elution at a flow rate of 0.2 ml · min^{-1}. The mobile phase was a multistep linear solvent gradient system, starting from 100% H_2O (adjusted to pH 3.2 by HCOOH) up to 100% CH_3CN in 35 min.

The response of soil microorganisms to *Solanum elaeagnifolium* densities

Microorganisms

Sixty samples represented high and low density (*HD* and *LD*) adjacent stands of *S. elaeagnifolium* (*HD* ≥ 10 plants · m^{-2} and low *LD* ≤ 10 plants · m^{-2}) in the two investigated areas (Borg El-arab and El-Hammam). Five grams were taken from rhizosphere soil samples, stored at –4°C and subsequently used for microbiological analysis. To enumerate the most important groups of soil fungi and bacteria, the dilution plate technique (Johnson *et al.* 1960) was used. Potato dextrose agar (PDA) media and nutrient agar (NA) were used for the enumeration of fungi and bacteria, respectively. Five Petri dishes were incubated at 25 ± 1°C for 5 days for fungi and at 30 ± 1°C for 24 h for bacteria (Parkinson *et al.* 1971). Data from five readings of replicates were expressed as Colony Forming Units (CFU) · g^{-1} soil.

Soil phenols

During the flowering stage of *S. elaeagnifolium*, a total of 60 samples was taken from rhizosphere and non-rhizosphere soil from two investigated areas (Borg El-arab and El-Hamamm). About 5 gm soil from each sample was taken for extraction with aqueous methanol by shaking for 24 h for phenolic analysis (Chiang *et al.* 2006). After centrifugation, the filtrate was subject to further purification by a resin column containing 5 g from Styrene-divinylbenzene Copolymer (Sigma-Aldrich) and eluted by ethyl acetate (Martens 2002). The elute was dried and dissolved to determine total phenols using Folin-Ciocalteu2 (FC) Colorimetric methods.

Statistical analysis

Data before analysis were checked for assumptions of homogeneity of variance and normality. Three-way ANOVA was done to test the main effects of factors of the plant part extract, concentrations and target species and interactions (Tables 1 and 2). Two-way ANOVA was carried out to separate the effect of extracts and concentration (Tables 3 and 4). Two-way ANOVA was done to separate two factors: the effect of extracts and concentration on soil properties response. The differences in the parts allelopathy, the concentration, and parameters among target species were compared using multiple comparisons. One-way ANOVA was done to separate the effect of root exudates on soil microorganisms (Table 5) using Dunnett's test. Tukey test based on least significant difference (LSD) values ($p > 0.05$) indicated the differences between treatments using SPSS, 19 software (SPSS, Chicago, IL USA) (Ott and Longnecker 2001).

Results

Efficacy of *Solanum elaeagnifolium* water extracts on some winter crops and weeds

Solanum elaeagnifolium aqueous extracts exerted a drastic reduction in the recipient plants' height and the length of shoots and roots and consequently seed germination.

Crops

The dose-response relationship of *S. elaeagnifolium* vegetative part extracts was exhibited by EC_{50} values of 2.06 and 2.25 *H. vulgare* as the most susceptible crop followed by 2.55 and 2.78 against *A. cepa* g DW (dry weight) · 100 ml^{-1} of root length and shoot length, respectively. While the EC_{50} values were 4.04 and 4.3 on *M. sativa* and 4.29 and 4.61 on *Z. mays* of root length and shoot length, respectively. However, the low sensitive crop which achieved EC_{50} values were 5.71and 5.97 on *V. faba* g DW · 100 ml^{-1} of root length and shoot length, respectively. Regarding root extracts, the calculated EC_{50} of the highest susceptible crop was 2.14 and 2.64 g DW · 100 ml^{-1} on *H. vulgare* in root length and shoot length, respectively. While the EC_{50} values were 4.31 and 4.4 g DW · 100 ml^{-1} on *M. sativa* and 5.12 and 5.24 g DW · 100 ml^{-1} on *Z. mays* of root and shoot length, respectively. However, the EC_{50} of the

lowest sensitive crop was recorded as 5.60 and 5.78 g DW · 100 ml^{-1} on *V. faba* in germination, root length and shoot length, respectively (Tables 1 and 2).

Weeds

Regarding the response of weeds to extracts from vegetative parts, the effective half dose of the highly susceptible weed was 1.99, 1.55 and 1.89 g DW · 100 ml^{-1} of *P. monospeliensis* germination, root length and shoot length, respectively. Among the tested weeds, EC_{50} values were 33.0, 215 and 2.19 (vegetative), 2.39, 2.17 and 2.22 (subterranean) g DW · 100 ml^{-1} of *P. minor*; 3.20, 2.47 and 2.64 (vegetative) and 3.81, 2.86 and 3.50 g DW · 100 ml^{-1} (subterranean) of *A. fatua*; 5.25, 3.23, 4.00 g DW · 100 ml^{-1} (vegetative), 5.70, 3.77, 4.47 (subterranean) extracts of *P. oleracea* and 4.56, 3.30 and 3.49 (vegetative), 4.60, 3.49 and 3.54 (subterranean) extracts of *C. arvensis* seeds germination, root length and shoot length, respectively. However, *C. olitorius* attained the lowest sensitivity with EC_{50} of 7.79, 5.25, 5.86 (vegetative), 7.66, 5.56, 5.94 (subterranean) extracts in seed germination, shoot and root length, respectively.

Multivariate analysis clarified the relative allelopathic potential of vegetative and root extracts in tested plants and traits. It showed that root length was the most susceptible trait to both extracts [(F = 244.035, $p \leq 0.00$) crops, (F = 46.759, $p \leq 0.00$) weeds and (F = 244.93, $p \leq 0.00$)] all plants, respectively. For root length the interactions between the extracts, concentrations and tested plants were significant [(F = 49.36, $p \leq 0.000$) crops, (F = 12.16, $p \leq 0.00$) weeds and (F = 56.89, $p \leq 0.000$)] for all tested plants, respectively. Therefore, it is possible to produce a tentative ranking of *S. elaeagnifolium* parts allelopathy as follows; vegetative plant parts were more highly phytotoxic than root parts extracts. As for the target plant traits sensitivity to *S. elaeagnifolium* allelochemicals, root length was the most sensitive, followed by shoot length and finally, seed germination was the weakest (Tables 1 and 2).

Comparison of *Solanum elaeagnifolium* vegetative (shoot), subterranean (root), mucilage, exudates extracted by ethyl acetate in seedling total biomass

To compare the four extracts, after water extraction by ethyl acetate we used a series of concentrations (0, 62.5, 125, 250, 500 and 750 µg · ml^{-1}) and *T. aestivum, T. alexandrinum, C. arvensis* seedlings. Based on the reduction percentage of total seedling fresh weights, vegetative parts had higher allelopathic efficiency than subterranean (root) parts. Moderate efficiency was achieved from mucilage extracts. Nevertheless, root

Table 1. Dose-response relationship (EC_{50} ± SD) of *Solanum elaeagnifolium* Cav. water extract on summer and winter crops and weed seed germination and seedling growth

Plant types	Species	Vegetative [g DW · 100 ml^{-1}]			Subterranean [g DW · 100 ml^{-1}]		
		germination	root length	shoot length	germination	root length	shoot length
Winter crops	*Triticum aestivum*	3.28 ± 0.33	2.79 ± 0.36	3.16 ± 0.42	3.85 ± 0.37	2.22 ± 0.53	3.27 ± 0.43
	Hordeum vulgare	4.140 ± 0.27	2.06 ± 0.07	2.25 ± 0.09	4.48 ± 0.23	2.14 ± 0.29	2.64 ± 0.38
	Trifolium alexandrinum	3.06 ± 0.52	2.31 ± 0.56	2.46 ± 0.22	3.69 ± 0.50	3.36 ± 0.48	3.65 ± 0.26
	Vicia faba	5.00 ± 0.30	5.71 ± 0.59	5.97 ± 0.33	5.09 ± 0.69	5.60 ± 0.64	5.79 ± 0.42
	Allium cepa	2.57 ± 0.01	2.55 ± 0.07	2.78 ± 0.06	2.92 ± 0.26	2.78 ± 0.33	2.88 ± 0.36
Summer crops	*Medicago sativa*	4.89 ± 0.64	4.04± 0.169	4.3 ± 0.231	5.2 ± 0.728	4.31 ± 0.2	4.4 ± 0.11
	Zea mays	5.88 ± 0.29	4.29 ± 0.345	4.61 ± 0.1	7.73 ± 0.86	5.12 ± 0.47	5.24 ± 0.2
Winter weeds	*Avena fatua*	3.20 ± 0.14	2.47 ± 0.19	2.64 ± 0.08	3.81 ± 0.80	2.86 ± 0.39	3.90 ± 0.57
	Polypogon monospeliensis	1.99 ± 0.29	1.55 ± 0.13	1.89 ± 0.34	2.63 ± 0.38	1.88 ± 0.58	1.95 ± 0.81
	Phalaris minor	2.33 ± 0.19	2.15 ± 0.02	2.19 ± 0.05	2.39 ± 0.25	2.17 ± 0.46	2.22 ± 0.31
	Convolvulus arvensis	4.56 ± 0.21	3.30 ± 0.13	3.49 ± 0.24	4.60 ± 0.24	3.49 ± 0.12	3.54 ± 0.43
Summer weeds	*Corchorus olitorius*	7.79 ± 0.126	5.25 ± 0.21	5.86 ± 0.26	7.66 ± 0.286	5.56 ± 0.20	5.94 ± 0.303
	Portulaca oleracea	5.25 ± 0.32	3.23 ± 0.32	4.00 ± 0.17	5.70 ± 0.13	3.77 ± 0.22	4.47 ± 0.31
	F	3.56	7.59	4.74	2.56	5.31	3.17
	$LSD_{(0.05)}$	1.08	0.35	0.54	1.063	0.65	0.76

EC_{50} – half maximal effective concentration; SD – standard deviation; g DW · 100 ml^{-1} – gram dry weight · 100 ml^{-1} of water

Table 2. The statistical analysis of *Solanum elaeagnifolium* Cav. phytotoxic effect on summer and winter crops and weed seed germination and seedling growth

Factors	Parameters	*F* (Vegetative × Subterranean extracts)					
		crops		weeds		all plants	
		F	(*p* value)	*F*	(*p* value)	*F*	(*p* value)
Extracts	germination	54.93	(0.00)	12.82	(0.042)	35.67	(0.000)
	root length	244.03	(0.00)	46.75	(0.00)	244.93	(0.000)
	shoot length	240.36	(0.00)	37.61	(0.00)	208.20	(0.000)
Target plants	germination	5.186	(0.025)	10.55	(0.04)	48.93	(0.000)
	root length	22.25	(0.00)	31.37	(0.000)	59.53	(0.000)
	shoot length	61.43	(0.00)	1.11	(0.294)	103.98	(0.000)
Concentration	germination	665.68	(0.00)	458.12	(0.00)	1,090.34	(0.000)
	root length	542.16	(0.00)	112.43	(0.00)	1,158.10	(0.000)
	shoot length	467.4	(0.00)	111.06	(0.00)	926.33	(0.000)
Extracts × × Target plants	germination	44.77	(0.00)	4.45	(0.00)	26.50	(0.00)
	root length	1.64	0.167	12.16	(0.00)	4.57	(0.00)
	shoot length	6.45	(0.00)	5.73	(0.00)	8.43	(0.00)
Extracts × Concentration	germination	38.31	(0.00)	6.90	(0.00)	38.13	(0.00)
	root length	8.57	(0.00)	0.684	0.63	7.83	(0.00)
	shoot length	5.44	(0.00)	2.87	0.018	13.37	(0.00)
Concentration × × Target plants	germination	20.15	(0.00)	4.45	(0.00)	14.75	(0.00)
	root length	49.3	(0.00)	12.16	(0.00)	56.88	(0.00)
	shoot length	34.67	(0.00)	5.73	(0.00)	29.78	(0.00)
Extracts × Concentration × × Target plants	germination	20.15	(0.00)	4.45	(0.00)	14.76	(0.000)
	root length	49.36	(0.00)	12.16	(0.00)	56.89	(0.000)
	shoot length	34.67	(0.00)	5.73	(0.00)	29.78	(0.000)

exudate extract was the least efficient in reducing the seeding weights of these plants. The interaction effects between extracts and concentration were significant in *T. aestivum* ($F = 60.77$, $p \leq 0.001$), *T. alexandrinum* ($F = 11.22$, $p \leq 000$) and *C. arvensis* ($F = 35.90$, $p \leq 0.03$) respectively, for the total biomass fresh weight of the tested plants, *T. alexandrinum* seems to be the most susceptible plant (Table 3).

Effects of *Solanum elaeagnifolium* on soil properties

Under greenhouse conditions, the effect of *S. elaeagnifolium* on soil pH and EC properties was slightly lower than the effect of *T. aestivum* on germination and growth. Despite these effects, there was a higher soil response to extracts of vegetative parts than to root part extracts, followed by the effect of mucilage extracts. However, root exudates have a slight effect on both vegetative indices and the tested soil properties. The interactions between plant parts and concentrations were affected significantly ($F = 12.13$, $p = 0.000$), vigour index ($F = 9.75$, $p = 0.001$), pH ($F = 5.5$, $p = 0.002$) EC, respectively (Table 4).

Quali-quantitative data of *Solanum elaeagnifolium* extracts

The allelochemical constituents were determined by comparison of mass spectra with those of authentic standards and bibliographic data using LC-DAD-MS (Fig. 1). The polyphenolic derivatives were in the range of 0.211–5.7 mg · g^{-1} for the analyzed samples. Also, flavonol constituents were found in the range of 0.00–2.392 mg · g^{-1} for the analyzed samples. In particular, in the vegetative extracts, apigenin, chlorogenic acid and cinnamic acid were the most abundant compounds, 1.650, 5.700, 3.269 mg · g^{-1}, respectively. In the root part extracts, cinnamic acid, quercetin, apigenin, and naringen were the most abundant flavonols (2.518, 1.848, 1.716, 1.356 mg · g^{-1}, respectively) followed by daidazin and luteolin, 1.272, 1.236 mg · g^{-1}, respectively. In root exudate extracts the most abundant were cinnamic acid, ferulic acid, p-coumaric acid and caffeic acids, 0.423, 0.254, 0.245, 0.242 mg · g^{-1}, respectively, followed by chlorogenic acid and quinic acid, 0.217, 0.211 mg · g^{-1}, respectively. Regarding mucilage extracts, chlorogenic acid, naringen and cinnamic acid, 2.661, 2.192, 1.149 mg · g^{-1}, respectively, were the most abundant (Table 5).

Table 3. Comparison of *Solanum elaeagnifolium* Cav. vegetative, root, mucilage and exudates extracted by ethyl acetate on the total biomass weight of the target plants

Species	Extract types	EC_{50} [µg · ml^{-1}]	Statistical analysis				
			extract types		concentration		extract types × × concentration
			F (*p* value)	$LSD_{(0.05)}$	*F* (*p* value)	$LSD_{(0.05)}$	*F* (*p* value)
Triticum aestivum	vegetative extracts	124.90 ± 0.373	448.19 (0.00)	0.095	3,189.2 (0.00)	0.61	60.77 (0.001)
	subterranean extracts	140.00 ± 0.367					
	mucilage	445.80 ± 0.25					
	exudates	778.36 ± 0.42					
Trifolium alexandrinum	vegetative extracts	125.04 ± 0.38	121.30 (0.00)	0.025	460.3 (0.00)	0.013	11.22 (0.00)
	subterranean extracts	125.22 ± 0.41					
	mucilage	449.79 ± 0.581					
	exudates	770.35 ± 0.432					
Convolvulus arvensis	vegetative extracts	115.62 ± 0.16	522.5 (0.00)	0.089	1367.9 (0.00)	0.31	35.90 (0.03)
	subterranean extracts	15.91 ± 0.211					
	mucilage	448.29 ±1.6					
	exudates	649.54 ± 0.41					

EC_{50} – the median effective concentration

Table 4. Influences of *Solanum elaeagnifolium* on vigor index of *Triticum aestivum* and soil parameters

Conc. [µg · ml^{-1}]	Vegetative parts extract			Subterranean parts extract			Mucilage extract			Root exudates extract		
	vigor index	pH	EC	vigor index	pH	EC	vigor index	pH	EC	vigor index	pH	EC
0.00	14,850.0	6.82	0.69	12,600.0	6.76	0.71	14,850.0	6.88	0.69	9,720.0	6.76	0.72
25	11,440.0	6.93	0.70	11,970.0	6.83	0.71	12,870.0	6.96	0.69	9,180.0	6.84	0.72
50	4,320.0	7.50	1.03	9,360.0	6.92	0.87	9,600.0	7.00	0.74	7,680.0	6.85	0.71
100	2,450.0	7.80	1.14	6,160.0	7.30	0.90	5,040.0	7.20	0.78	5,775.0	6.89	0.77
200	2,275.0	7.90	1.16	4,275.0	7.45	0.97	3,360.0	7.30	0.86	4,900.0	6.93	0.80
F	*F* = 33.2 *p* = 0.00	*F* = 5.00 *p* = 0.012	*F* = 8.00 *p* = 0.001	*F* = 21.2 *p* = 0.00	*F* = 9.00 *p* = 0.002	*F* = 4.50 *p* = 0.006	*F* = 17.2 *p* = 0.00	*F* = 3.5 *p* = 0.042	*F* = 4.84 *p* = 0.031	*F* = 13.5 *p* = 0.027	*F* = 2.08 *p* = 0.153	*F* = 3.00 *p* = 0.045
$LSD_{(0.05)}$	3,670.54	0.18	0.23	936.50	0.34	0.12	853.00	0.17	0.05	615.00	NS*	0.02
Interaction (Plant part × × Conc.)	Vigor index						*F* = 12.13, *p* = 0.000					
	pH						*F* = 9.75, *p* = 0.001					
	EC						*F* = 5.5, *p* = 0.002					

*NS – non significant; Conc. = Concentration; EC – electrical conductivity

Total phenols in the rhizosphere and non-rhizosphere soils of the two invaded areas

Total phenol content was determined calorimetrically in the soil samples collected from the invaded natural habitats at Borg El-arab and El Hammam localities in both rhizosphere and non-rhizosphere soil. The total phenols in rhizosphere samples reached 0.956 to 0.987 mg · g^{-1}, while in non-rhizosphere soil it was 0.312 to 0.325 mg · g^{-1} soil. The concentrations of these phenolic compounds were higher in El-Hammam than in Borg El-arab (Fig. 2). The differences in phenol quantity were higher in the rhizosphere than in non-rhizosphere soil. These results could be attributed to the higher infestation and the successful establishment of *S. elaeagnifolium* in El-Hammam localities than Borg El-arab.

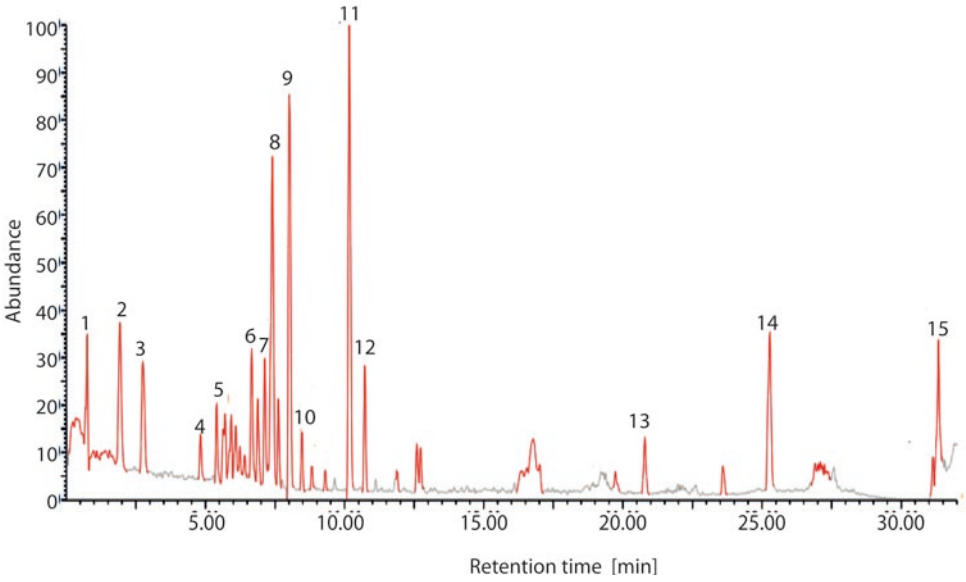
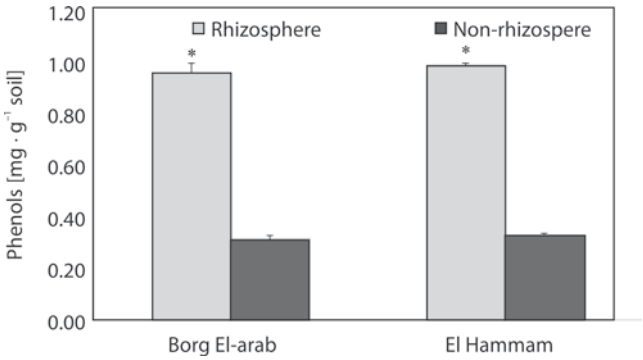


Fig. 1. Chromatographic profiles of the tentatively identified molecules of *Solanum elaeagnifolium* presented in Table 4

Studying the impact of *Solanum elaeagnifolium* on associated soil microbes

The variations in total counts were even greater within fungi and bacteria as well as among the investigated regions and density. At high density (HD), a greater reduction was observed in the total count of fungi than in bacteria and in El-Hammam than Borg El-arab region. Nevertheless, small impacts in both total counts of fungi and bacteria were detected at low density (LD). The decrease in total microbial counts was even greater in HD than LD areas and in fungi than in bacteria in the rhizosphere soils. It would be assumed that a remarked significant suppression was detected in fungi counts than bacteria, especially in HD of soil rhizosphere associated with *S. elaeagnifolium*. The interaction effects of *S. elaeagnifolium* densities and invaded region were significantly ($F = 4.04$, $p \leq 0.03$) bacteria and ($F = 5.46$, $p \leq 0.02$) fungi respectively (Table 6).



*statistical analysis (significant)

Fig. 2. Total phenols in the rhizosphere and non-rhizosphere soil of *Solanum elaeagnifolium* in Borg El-arab and El Hammam localities

Discussion

The allelopathic activity of *Solanum elaeagnifolium* against broad spectrum plants

Allelopathy is an invasion mechanism. Extensive screening of invasive *S. elaeagnifolium* allelopathic potentials of extracts of different organs (vegetative, root, exudates and mucilage) were tested against many monocot and dicot plant species. Dose-response of extracts could potentially cause the observed seed germination and growth reductions proportional to the used concentrations toward broad-spectrum species. It was found that root length was the most sensitive trait to allelochemicals. Also, monocots appeared to be a more susceptible crop than other receivers and dicot crops. As a consequence, *H. vulgare* was the most sensitive and *V. faba* appeared to be the least sensitive to all extracts. Among weeds, *P. monospeliensis* was the most sensitive. These results agree with Mkula (2006) who found that *S. elaeagnifolium* fruits have allelopathic effects on several crops such as cotton and cucumber and pasture establishments due to their toxic saponins. Germination and early growth of cotton were inhibited by extract solutions and soil-incorporated residues of *S. elaeagnifolium*. The allelopathic effects primarily

Table 5. Qualitative-quantitative composition of the analyzed *Solanum elaeagni Folium* Cav. samples

No.	Rt	Compounds	$[M+1]^+$	$[M-1]^-$	Frag. ESI	Vegetative [mg · g^{-1}]	Subterranean parts [mg · g^{-1}]	Root exudates [mg · g^{-1}]	Mucilage [mg · kg^{-1}]
1.	0.75	gallic acids	171	169	125, 152	1.375 ± 0.13	0.884 ± 0.02	0.395 ± 0.00	0.224 ± 0.00
2.	1.94	quinic acid	193.1	191.1	215, 256	1.245 ± 0.03	0.390 ± 0.06	0.211 ± 0.04	0.295 ± 0.00
3.	2.75	caffeic acids	181	179	147, 135	1.257 ± 0.14	0.782 ± 0.02	0.242 ± 0.05	0.639 ± 0.07
4.	5.41	ferulic acid	195	193	194, 176	1.573 ± 0.10	0.294 ± 0.04	0.254 ± 0.03	1.318 ± 0.10
5.	6.65	chlorogenic acid	355	354	269, 163	5.700 ± 0.12	1.785 ± 0.13	0.217 ± 0.01	2.661 ± 0.14
6.	7.40	p-coumaric acid	164	164	147, 125	1.575 ± 0.04	0.518 ± 0.08	0.245 ± 0.03	0.332 ± 0.00
7.	7.60	cinnamic acid	149	147	132, 103	3.269 ± 0.16	2.518 ± 0.18	0.423 ± 0.08	1.149 ± 0.07
8.	8.03	quercetin	303	301	149, 153	2.261 ± 0.13	1.848 ± 0.14	0.176 ± 0.01	0.602 ± 0.04
9.	8.47	apigenin	271	269	303, 153	3.011.650 ± 0.11	1.716 ± 0.10	0.164 ± 0.00	0.766 ± 0.02
10.	9.34	naringen	273	271	185, 151	1.342 ± 0.02	1.356 ± 0.08	0.02 ± 0.00	2.192 ± 0.13
11.	10.26	luteolin	287	285	151, 179	1.290 ± 0.05	1.236 ± 0.09	0.000 ± 0.00	0.823 ± 0.05
12.	12.54	kaempferol	287	285	153, 165	2.392 ± 0.08	1.492 ± 0.12	0.310 ± 0.05	1.338 ± 0.15
13.	20.79	isorhamnetin	316	316	255, 273	0.792 ± 0.03	0.552 ± 0.04	0.000 ± 0.00	0.616 ± 0.08
14.	25.28	myricetin	319	317	149, 130	0.583 ± 0.01	0.259 ± 0.01	0.141 ± 0.00	1.378 ± 0.06
15.	31.13	daidazin	417	417	255, 133	1.318 ± 0.14	1.272 ± 0.10	0.000 ± 0.00	0.812 ± 0.04
		F ($p < 0.05$)				7.95 (0.00)	4.40 (0.00)	2.19 (0.23)	2.83 (0.02)
		$LSD_{(0.05)}$				0.823	0.954	NS	0.697

Rt – retention time; M – molecular ions; Frag. ESI – fragments produced from electrospray ionization in mass spectrometry; ± – standard deviation

Table 6. Effect of *Solanum elaeagnifolium* on rhizosphere bacteria and fungi total counts in Borg El-arab and El-Hammam regions

Regions	Fungi total counts (× 10^{-2})		Bacteria total counts (× 10^{-5})	
	high density [>20 m^2]	low density [<20 plants · m^{-2}]	high density [>20 m^2]	low density [<20 plants · m^{-2}]
Borg El-arab	0.99 ± 0.75	3.94 ± 1.35	10.27 ± 1.97	16.76 ± 3.03
El-Hammam	0.1 ± 0.14	2.36 ± 0.96	6.08 ± 0.82	13.57 ± 2.04

Soil microbes		Statistical analysis					
		interaction (region × density)		density		region	
		high	low	high	low	high	low
Bacteria	F	4.04	2.66	8.64	7.90	4.14	5.87
	p value	0.03	0.08	**0.00**	**0.00**	0.04	0.03
Fungi	F	5.46	3.41	8.88	0.49	13.11	3.61
	p value	0.02	0.05	**0.00**	0.74	0.005	0.05

varied with the target weeds. Root system length was the most sensitive for all weed species (Scavo *et al.* 2019a). With increases in the concentration of water extracts, stronger inhibitory effects were found (Hang *et al.* 2020). From the multivariate analysis it was clear that vegetative extracts had the greatest reduction activity followed by extracts of root parts in crops and weeds. *S. elaeagnifolium* water extracts had more remarkable effects on root length than other growth parameters. Mucilage extract had moderate allelopathic potential. In contrast, root exudate extracts were the least phytotoxic on the parameters of the tested plants. There was greater effectiveness and broad-spectrum suppressive abilities of *S. elaeagnifolium* against certain monocot and dicot species based on the used part and concentrations. In general, the effectiveness of

S. elaeagnifolium was diverse across the types of extracts, concentrations, and received species. These results are supported by Bothma (2002) who reported that the allelopathy by water-soluble extracts of *S. elaeagnifolium* foliage inhibited germination and root growth of cotton and lettuce, respectively. Invasive species leachates may be harmful to native plants (Zheng *et al.* 2017). *Solanum elaeagnifolium* foliage powders were more effective in preventing germination and growth of wheat than root powders (Alhemedy *et al.* 2016).

The possible role of allelopathy in *Solanum elaeagnifolium* invasion

The great invasive capacity of *S. elaeagnifolium* weeds in new agroecosystems could be explained by their allelochemical interactions with the soil and subsequently their influence on soil properties based on their chemical and biological characteristics. These influences on soil properties of pH, EC, microorganisms and phenols were perceived on different scales relative to the extract types and concentrations and densities of *S. elaeagnifolium* in natural habitats. The soil properties were slightly affected by these extracts when compared with the response of recipient plants. The pH value is one of the most important factors affecting the availability of nutrients in the soil, while EC values directly affect plant germination and growth. In natural habitats the detected, phenolic allelochemicals were high, especially in a higher density of *S. elaeagnifolium* which may negatively affect the soil or make the surrounding environment unsuitable for other plant communities. This potential of *S. elaeagnifolium* may cause remarkable interactions which change the recipient community and soil responses in natural settings. These illustrate the role of *S. elaeagnifolium* allelochemicals in their invasion that may act as biochemical mediators to change the habitat characteristics to be less suitable for the native species. These results are supported by Ambika (2013) and Scavo *et al.* (2018) who found that higher concentrations displayed higher phytotoxic effects. Invasive weeds may exert a negative impact on other plant species driven by allelopathy (Majumdar *et al.* 2017). The response of native species to invasive species can be explained by allelochemical and biochemical mediators (Baležentienė 2015). It may be one or more joint activities of allelochemicals that participate in their facilitation of invasion. Root exudate substances that affect the growth of crop species may change the soil conditions and adversely affect other species. These results are in agreement with Boyd *et al.* (1984) who reported that *S. elaeagnifolium* exudes plant inhibitors. *Solanum elaeagnifolium* is a pioneer plant that appears to be adapted to a wide range of habitats, different soil types and conditions (Parsons 1981; Heap and Carter 1999). This ability can facilitate their establishment in new areas. Flavonoids are exuded from some Solanaceae species (Wollenweber and Dorr 1995). Allelopathy helps to explain the success of an invasion (Uddin *et al.* 2017). Allelopathic effects can regulate the invasion of plants (Chen *et al.* 2017). Allelopathy of indigenous plant communities may increase their resistance to introduced plants (Ning *et al.* 2016). Successful invaders are released allelopathic compounds that are highly suppressive to native competitors in invaded ranges (Oduor *et al.* 2020). Accordingly, the vegetative extract was found to be more active than root part extracts, followed by mucilage extracts which showed greater potential than root exudates in plant growth and soil properties. The prospective potentials of *S. elaeagnifolium* were not natural habitats. Despite the complicated interactions in natural habitats, it is possible to infer the higher interference abilities of *S. elaeagnifolium* via its allelopathic properties. These results are supported by Scavo *et al.* (2019b) who reported that allelochemical activity in the soil is affected by climatic conditions, soil factors (e.g. texture, pH, ion-exchange capacity, organic matter content, nutrient dynamics, moisture content and microbial ecology) and plant factors of both the donor and target plants. The allelochemicals interact with the organic and inorganic soil phases, as well as with soil microorganisms. Accordingly, these chemical interference abilities are the possible reasons for the successful invasion in many localities. These outcomes were confirmed by LC-MS whereas *S. elaeagnifolium* parts contain high amounts of bioactive constituents. The greatest depressing impact on the tested dicot and monocot germination and seedling growth is evident of *S. elaeagnifolium* aggressiveness and exploitation of the environmental resources in natural habitats.

Proportional *Solanum eleaegnifolium* allelochemical potential and the possible use in agriculture

Several phytotoxins have been isolated from *Solanum* sp. in prior research. Here we used aqueous and ethyl acetate extracts. The content of these extract substances can vary according to the plant parts, extract type and polarity. The most abundant constituent in the vegetative part extracts was chlorogenic acid (5.700 $\mu g \cdot g^{-1}$). Cinnamic acid and quercetin, (2.518, 1.848 $\mu g \cdot g^{-1}$) were the most abundant in the root part extracts, and cinnamic acid and gallic acid (0.423, 0.395 $\mu g \cdot g^{-1}$) were the most abundant in root exudates and chlorogenic acid (2.661 $\mu g \cdot g^{-1}$), in mucilage extracts (Table 4). It is hard to specify the functioning compound(s) that inhibits seed germination and seedling growth of other plants particularly in different soil types and environmental conditions. These phytotoxic

constituents may play a vital role in *S. elaeagnifolium* invasion or facilitate their success to invade new areas. The allelochemicals can change in response to environmental stress (Einhellig 1996). Chlorogenic acids and kaempferol 3β-D-(6-O-cis-cinnamoyl glucoside) are the most active compounds responsible for the allelopathic effects of *S. elaeagnifolium* fruits (Balah 2015, 2020). There are no selective actions within the target tested crops. Allelochemicals of phenolic compounds are effective against weeds and are relatively non-selective (Duke and Lydon 1993). However, in a few cases some allelochemicals have selective herbicidal action such as artemisinin and its potency could be a possible additional advantage to consider using the chemical as a potential natural herbicide (Duke *et al.* 1987; Chen and Leather 1990). On the other hand, abiotic and biotic conditions should be considered in evaluating the effectiveness of allelopathy in natural settings (Li *et al.* 2017). Allelopathy is the reason for the rapid displacement of native species (Bais *et al.* 2003) and a mechanism of the success of invaders (Hierro and Callaway 2003). Therefore, allelopathy has broad agricultural and ecological applications (Nelson 1996). Allelopathy is attributed to the success of an invasive species in natural ecosystems (Kimura *et al.* 2015). Allelopathy is known to affect individual performance, community structure and plant invasions (Zhang *et al.* 2020). Allelopathic substances can inhibit the germination and growth of neighboring plants and may enhance the competitive ability of the plants, making them invasive (Kato-Noguchi 2020).

Conclusions

The allelopathic potential of invasive *S. elaeagnifolium* weed on many plant species, including monocots and dicots, was shown in the above experiments. The LC-MS analysis has remarkable phytotoxic constituents in all parts that can retard the growth and development of exposed plants. Allelopathic abilities were diverse across extract types, concentrations, and species. The relative phytotoxicity on the measured parameters was the highest in vegetative extracts, followed by root parts and mucilage extracts. Root exudates had a slight reduction in seedling growth. Despite the great phytotoxic activity of *S. elaeagnifoliumn* extracts in inhibiting the plant growth, it had a weak effect on soil properties. However, it had a significant effect on soil microorganisms especially in low densities of *S. elaeagnifoliumn*. Accordingly, these vast arrays of secondary metabolites of *S. elaeagnifolium* parts get into the environment and cause serious threats to crop production and ecosystem biodiversity and may help in breaking the barriers that face their invasion. Consequently, S. *elaeagnifolium* should be managed and all parts removed carefully from infected land to maximize productivity and to limit the spreading and invasion to other land. On the other hand, these activities can be utilized through pronounced allelochemicals that may be developed as natural herbicides against several weed species in the future.

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