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Argemone ochroleuca Phytochemicals and Allelopathic Effect of Their Extracts on Germination of Soybean

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Abstract: Soybean is a high-value food source, and the invasive weeds Mexican prickly poppy (*Argemone ochroleuca*) could release allelochemicals that inhibit the growth of this crop. The impact of *A. ochroleuca* on the germination and growth of soybean is not well documented. Therefore, the aim of this study was to evaluate the TLC profiles of different extracts of *A. ochroleuca* and assess the effects of extracts on the germination of soybean seeds. Shoots and roots of *A. ochroleuca* were weighed and 100 g of each was separately extracted with 1000 mL deionized water, hexane or acetone. Ten concentrations of water extracts ranging from 10 to 100 mL per 100 mL of deionized water and three concentrations of acetone and hexane extracts ranging from 2.5 to 7.5 g/L were separately used for seed germination bioassays. Thin-layer chromatography (TLC) analysis was used to compare the chemical profiles in the shoot and root water, and in the hexane and acetone extracts of *A. ochroleuca*. The highest reduction was recorded from the water extract, at 100%. The TLC profiling of *A. ochroleuca* addressed different classes of compounds, including alkaloids, phenolic acids and flavanoids. There is, however, a need to identify the most active phytochemicals in the suppression of germination.

Keywords: inhibition; invasive alien weeds; Mexican prickly poppy; phytochemistry; phytotoxicity

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1. Introduction

Invasive alien weeds are a major problem in agriculture, reducing crop yields [1]. *Argemone ochroleuca* (Sweet) is among the most important economically devastating invasive plant species that affect both agricultural and natural ecosystems [2]. This weed has been reported to release allelochemicals that affect crops in agricultural fields [3,4]. Muche et al. [4] observed that *A. ochroleuca* leaf, stem and root, and water extracts inhibited the growth and germination of three sorghum varieties. Abd-ElGawad et al. [5] reported that the chemical composition of *A. ochroleuca* essential oils had highly oxygenated constituents including mono-, sesqui-, di-terpenoids, carotenoids, and hydrocarbons. These compounds have been associated with the inhibition of the germination and growth of crops [5]. Few empirical studies have been conducted to assess the impact of *A. ochroleuca* on crops in South African agricultural fields, leaving the effects speculative due to the limited research on the germination and growth of locally produced crops.

Soybean (*Glycine max* (L.) Merr.) is a high-value leguminous food source and an oil seed crop important for human consumption and animal feeds [6]. Khojely et al. [7] reported that in Africa, the soybean is a non-staple and non-native crop with the prospective to become a commercialized crop due to its multiple applications as food, industrial raw material, and feed. Soybean is also considered a significant source of oil and protein [8] and accounts for about a quarter of global protein and animal feed production [9]. In South Africa, soybean is mostly cultivated in the Free State, KwaZulu-Natal, Mpumalanga, and Gauteng Provinces, but its production is extended to areas where predominantly maize

and crops such as groundnut, sunflower, potato and others are traditionally grown [10]. The need for soybean is gradually increasing due to an increase in population and societal development [11]. However, the production of soybean is currently threatened by invasive alien weeds all over the world [12].

Paul and Begum [13] reported that the aqueous extracts of *A. mexicana* root and leaf could reduce the germination of *Lens culinaris*. This conforms to a study by Alagesaboopathi [14], who reported that *A. mexicana*, another *Argemone* species, decreased seed germination of *Sorghum bicolor* by 18% compared to the 89% germination observed in the control. Dar et al. [15] also noted that the shoot extracts of *A. ochroleuca* inhibited the germination of *Farsetia aegyptia* Turra, *Salvia aegyptiaca*, *Hordeum vulgare* and *Medicago sativa* more than their corresponding root extracts. With *A. ochroleuca* spreading across Africa at an alarming rate [2], the understanding of how this invasive species affects economic crops is the starting point in the management decisions [16]. Currently, there is no information on the impact of *A. ochroleuca* on the germination of soybean, one of the most economically important crops belonging to the Leguminosae family, and the information on the phytochemicals present in this plant is inadequate. Therefore, the aim of the current study was to evaluate the TLC profiles of different extracts of *A. ochroleuca* and determine the effects of shoot and root extracts on soybean seed germination.

2. Materials and Methods

2.1. Collection and Preparation of Argemone ochroleuca Plant Extracts

Argemone ochroleuca (flowering) plant materials were collected from the University of Mpumalanga farm (25.4365° S, 30.9818° E). The botanical identity of the plant was confirmed by the H.G.W.J. Schweickerdt Herbarium (PRU), Pretoria, South Africa and given a voucher number PRU 130499. The shoots and roots of the weed were separated and cleaned thoroughly with running tap water. The collected shoots and roots were placed inside brown paper bags and dried in an oven (Memmet UN 110, Lasec, Eagle, WI, USA) set at a temperature of 55 °C for 72 h. The dried plant materials were then ground in an electric grinder (BBS1200, Summit Pro Blend, China) and sieved into a conical flask using a 2 mm sieve.

2.2. Preparation of Crude Extracts

In total, 100 g of shoot and root extracts were separately extracted in 1000 mL of water, hexane and acetone.

Aqueous water extract: Extraction was performed using deionized water and the sample was left at room temperature (25 °C) for 24 h. Each mixture was filtered through a 2 µm sieve. The resultant aqueous supernatant was considered as a 100% concentration, and subsequent dilutions of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mL per 100 mL of deionized water were prepared by adding deionized water into 100 mL bottles as previously stated by Namkeleja et al. [17].

Organic solvents: The acetone and hexane mixtures were left at room temperature for 3 days with occasional stirring. The mixture was passed through a Whatman No. 1 filter paper with the resultant filtrates evaporated to dryness using the stream of cold air from the lamina flow. Acetone and hexane extract concentrations of 0, 2.5, 5 and 7.5 g/L were prepared from the roots and shoots. These dilutions were kept at room temperature for 24 h. The extracts were then centrifuged at 4000 rpm for 15 min and the supernatants were used in the germination test [18].

2.3. Germination Bioassay

Germination tests were conducted following the techniques of the International Seed Testing Association (ISTA) [19]. Briefly, soybean seeds (cultivar PAN 1532R-ZXG7) was purchased from Pannar Seeds, South Africa) were separately surface-sterilized for 1 min by soaking them in 3% sodium hypochlorite and then washing with deionized water two times each for 3 min. Ten seeds were placed in a 9 cm diameter Petri dish that was lined

with Whatman No. 1 filter paper. The seeds were treated with 10 mL water extracts from *A. ochroleuca* shoot or root concentrations; 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mL per 100 mL of deionized water was added using a syringe, whereas for hexane and acetone extract bioassays, seeds were separately treated with the four extract concentrations of 0, 2.5, 5 and 7.5 g/L of deionized water daily. All treatments were replicated three times and trials were duplicated three times for water extracts and two times for hexane and acetone extracts. The experiments were carried out in a growth chamber at room temperature (±25 °C) with 8 h of light for ten days with germination counts taken daily until the tenth day.

Seeds were considered as germinated when both the radicle and plumule were 2 mm long. On the tenth day, the last germination count was made along with the measurement of plumule and radicle lengths. Germination percentage, germination speed, mean germination time, mean daily germination and germination index were computed using formulae previously described by Gairola et al. [20]:

$$\text{Germination \%} = \frac{\text{Number of germinated seeds in each Petri dish}}{\text{Total number of seeds in each Petri dish}} \times 100 \quad (1)$$

$$\text{Germination speed} = \frac{n1}{d1} + \frac{n2}{d2} + \frac{n3}{d3} \pm - - - - - - - - - - \quad (2)$$

$$\text{Mean germination time} = \frac{n1 \times d1 + n2 \times d2 + n3 \times d3 + - - - - -}{\text{Total number of days}} \quad (3)$$

where n = number of germinated seed d = number of days

$$\text{Mean daily germination} = \frac{\text{Total number of seeds germinated}}{\text{Total number of days}} \quad (4)$$

$$\text{Germination index} = \frac{\text{Number of germinated seeds of day of first count}}{\text{Day of first count}} + \cdots \frac{\text{Number of germinated seeds of day of final count}}{\text{Day of final count}} \quad (5)$$

2.4. Thin Layer Chromatography (TLC) Analysis

Here, 2 g of each plant part was separately extracted in 20 mL of acetone, hexane and hot distilled water. The mixture was left at room temperature for 30 min and filtered through Whatman No. 1 filter paper. The procedure was repeated twice to exhaustively extract plant material, and the second collection was done after an hour. Filtrates were concentrated by drying in front of a stream of cold air to obtain crude extracts. The crude extracts were stored in an air-tight container at 25 °C until further use.

Chemical constituents in the acetone, hexane and water extracts were analyzed by thin-layer chromatography (TLC) using aluminum-backed TLC plates (ALIGRAM®SIL g/UV 254 MACHEREY-NAGEL, Merk, Johannesburg, South Africa). Then, 10 mg/mL of the extract redissolved in the respective solvents were loaded in bands of approximately 1 cm in length of the TLC plate. Plates were developed using three eluent systems developed by Kotze et al. [21]:

Ethyl acetate:methanol:water = 40:5.4:4 [EMW] (polar)

Chloroform:ethyl acetate:formic acid = 5:4:1 [CEF] (intermediate polar)

Benzene:ethanol:ammonium hydroxide = 90:10:1 [BEA] (non-polar/basic)

After development in the respective eluent system, the dried plates were visualized under ultraviolet light (254 and 366 nm, Camac Universal UV lamp TL-600). For the detection of compounds not visible under UV light, a vanillin–sulfuric acid spray reagent (1 g vanillin in 28 mL of methanol and 1 mL sulfuric acid) was used.

The retention factor was measured, which is defined as the ratio of the distance traveled by the center of a spot to the distance traveled by the solvent front.

R_f value = distance moved by the component from the origin to the spot center/distance moved from the origin by the solvent front.

2.5. Statistical Analysis

The obtained germination data were subjected to two-way independent (concentration × plant part) analysis of variance (ANOVA) through Statistix 10.0 software [22]. Pre-ANOVA, Shapiro–Wilk normality test were conducted to determine the normality of residual distribution [23]. All the variables that failed the normality test ($p \leq 0.05$) were transformed using $\text{Log}_{10}(x + 1)$, and $\text{arcsine}\sqrt{(x \div 100)}$ was used for percentage variables. The mean separation was achieved using Fisher's least significant difference at a 5% probability level.

3. Results

There were no statistical differences between trials, hence the data were pooled and reanalyzed as one data set. All data on the germination variables of soybean seeds treated with water, acetone, and hexane extracts were not normally distributed, except for germination speed and mean germination time of hexane extract and germination speed under acetone extract, hence data were transformed accordingly.

3.1. Effects of Argemone ochroleuca Water Extracts on Soybean Seed Germination

The interactions between water extract concentrations and plant part were highly significant ($p < 0.01$) for all germination variables and seedling length variables. Concentration and plant parts of *A. ochroleuca* as separate factors were also highly significant ($p < 0.01$) for all germination variables and seedling length variables. Increasing the concentrations of both root and shoot extracts significantly decreased all germination variables and seedling length variables (Tables 1 and 2). Root extract concentrations of 90–100, 70–100, 100, 90–100, 100, 30–100 and 20–100 mL per 100 mL of deionized water reduced germination percentage, germination speed, mean germination time, mean daily germination, germination index, plumule and radicle length, respectively. Shoot extract concentrations of 40 and above generally reduced germination variables and seedling length variables (Tables 1 and 2). In relation to the untreated control, *A. ochroleuca* root extracts decreased germination percentage (26–49%), germination speed (15–40%), mean germination time (29%), mean daily germination (24–45%), germination index (33%), plumule length (11–64%) and radicle length (15–94%) (Tables 1 and 2), whereas shoot extracts decreased germination percentage (39–85%), germination speed (32–84%), mean germination time (18–72%), mean daily germination (32–89%), germination index (20–79%), plumule length (15–95%) and radicle length (31–100%).

Table 1. Soybean seeds' germination response to *Argemone ochroleuca* water extracts.

Concentration (%)	Plant Part	Mean Germination Percentage		Mean Germination Speed		Mean Germination Time		Mean Daily Germination		Germination Index	
		Mean [x]	RI [y]	Mean	RI	Mean	RI	Mean	RI	Mean	RI
0	Roots	1.35 (90.00) ^{abc}	-	1.26 (17.29) ^{a}	-	1.68 (47.93) ^{ab}	-	0.28 (0.90) ^{abc}	-	1.03 (9.90) ^{ab}	-
0	Shoots	1.38 (90.00) ^{ab}	-	1.24 (16.76) ^{abc}	-	1.68 (47.84) ^{ab}	-	0.28 (0.90) ^{abc}	-	1.03 (9.90) ^{ab}	-
10	Roots	1.41 (94.44) ^{a}	4	1.26 (17.29) ^{a}	0	1.71 (49.87) ^{a}	1	0.29 (0.94) ^{a}	4	1.06 (10.39) ^{a}	2
10	Shoots	1.45 (95.56) ^{a}	5	1.25 (16.97) ^{ab}	1	1.71 (50.20) ^{a}	2	0.29 (0.96) ^{a}	5	1.06 (10.51) ^{a}	3
20	Roots	1.32 (87.50) ^{abc}	−3	1.19 (14.88) ^{abcd}	−5	1.66 (45.84) ^{ab}	−1	0.27 (0.88) ^{abc}	−2	1.02 (9.62) ^{abc}	−1
20	Shoots	1.39 (91.11) ^{a}	1	1.18 (14.43) ^{abcd}	−5	1.67 (46.76) ^{ab}	−1	0.28 (0.91) ^{abc}	1	1.04 (10.02) ^{ab}	1
30	Roots	1.35 (87.78) ^{abc}	−0	1.18 (14.57) ^{abcd}	−6	1.66 (45.49) ^{ab}	−2	0.27 (0.88) ^{abc}	−2	1.02 (9.66) ^{abc}	−1
30	Shoots	1.23 (80.00) ^{abcd}	−11	1.07 (11.53) ^{bcde}	−14	1.57 (38.90) ^{abc}	−7	0.25 (0.80) ^{abcd}	−9	0.97 (8.80) ^{abcd}	−6
40	Roots	1.42 (93.33) ^{a}	5	1.21 (15.52) ^{abcd}	−3	1.69 (48.10) ^{ab}	0	0.29 (0.93) ^{ab}	3	1.05 (10.27) ^{a}	2
40	Shoots	0.84 (55.56) ^{fgh}	−39	0.84 (6.64) ^{fg}	−32	1.38 (25.13) ^{cd}	−18	0.19 (0.56) ^{fg}	−32	0.83 (6.11) ^{de}	−20
50	Roots	1.37 (88.89) ^{abc}	1	1.15 (13.54) ^{abcd}	−9	1.64 (44.08) ^{abc}	−3	0.27 (0.89) ^{abc}	−1	1.02 (9.78) ^{abc}	−1
50	Shoots	0.93 (60.00) ^{efg}	−33	0.91 (7.70) ^{efg}	−27	1.44 (28.42) ^{bcd}	−15	0.20 (0.60) ^{def}	−28	0.85 (6.60) ^{cde}	−17
60	Roots	1.18 (75.56) ^{abcde}	−13	1.09 (12.58) ^{abcde}	−13	1.56 (39.01) ^{abc}	−8	0.24 (0.76) ^{abcde}	−15	0.93 (8.31) ^{abcd}	−10
60	Shoots	0.59 (35.56) ^{hij}	−57	0.61 (4.36) ^{hi}	−51	1.06 (16.13) ^{ef}	−37	0.12 (0.36) ^{gh}	−55	0.59 (3.91) ^{fg}	−42
70	Roots	1.10 (71.11) ^{bcdef}	−19	1.06 (11.43) ^{cde}	−15	1.53 (35.86) ^{abc}	−9	0.23 (0.71) ^{cde}	−18	0.92 (7.82) ^{abcd}	−11
70	Shoots	0.52 (27.78) ^{ijk}	−62	0.57 (3.17) ^{i}	−54	1.02 (12.26) ^{ef}	−39	0.10 (0.28) ^{gh}	−63	0.56 (3.06) ^{fg}	−46
80	Roots	1.08 (72.22) ^{cdef}	−20	1.05 (10.67) ^{de}	−16	1.54 (35.44) ^{abc}	−9	0.23 (0.72) ^{bcde}	−17	0.93 (7.94) ^{abcd}	−10
80	Shoots	0.40 (20.00) ^{jkl}	−71	0.44 (2.27) ^{i}	−65	0.84 (9.58) ^{f}	−50	0.08 (0.20) ^{hi}	−73	0.43 (2.20) ^{g}	−59
90	Roots	1.00 (65.56) ^{def}	−26	0.95 (8.96) ^{ef}	−25	1.45 (31.66) ^{abc}	−14	0.21 (0.66) ^{de}	−24	0.87 (7.21) ^{bcd}	−16
90	Shoots	0.24 (10.00) ^{kl}	−83	0.19 (0.75) ^{j}	−84	0.46 (3.76) ^{g}	−72	0.04 (0.10) ^{i}	−86	0.25 (1.10) ^{h}	−76
100	Roots	0.69 (44.44) ^{ghi}	−49	0.76 (5.96) ^{gh}	−40	1.19 (20.67) ^{de}	−29	0.15 (0.44) ^{fg}	−45	0.69 (4.89) ^{ef}	−33
100	Shoots	0.21 (7.78) ^{l}	−85	0.20 (0.82) ^{j}	−84	0.46 (3.69) ^{g}	−72	0.03 (0.08) ^{1}	−89	0.21 (0.86) ^{h}	−79
p-value		0.0000		0.0000		0.0000		0.0000		0.0000	

Table 1. *Cont.*

Concentration (%)	Plant Part	Mean Germination Percentage		Mean Germination Speed		Mean Germination Time		Mean Daily Germination		Germination Index	
		Mean [x]	RI [y]	Mean	RI	Mean	RI	Mean	RI	Mean	RI
F-value		4.56		8.26		6.67		5.67		6.54	
$LSD_{0.05}$		0.2890		0.1838		0.2617		0.0547		0.1743	

[x] Column means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's least significant difference. [y] Relative impact (%) = [(treatment/control) − 1] × 100. Values in brackets are untransformed means [arcsine(x/100)]/[Log (x + 1)].

Table 2. Effect of *Argemone ochroleuca* water extracts on soybean plumule and radicle length (mm).

Concentrations (%)	Plant Part								
	Plumule				Radicle			
	Shoots [x]	RI [y]	Roots	RI	Shoots	RI	Roots	RI
0	1.52 (54.21) ^{abc}	-	1.59 (58.43) ^{ab}	-	1.24 (34.84) ^{ab}	-	1.32 (40.69) ^{a}	-
10	1.67 (59.31) ^{a}	9	1.63 (56.43) ^{a}	2	1.32 (37.57) ^{a}	6	1.31 (30.18) ^{a}	−1
20	1.53 (52.07) ^{abc}	0	1.51 (52.87) ^{abc}	−5	1.21 (26.74) ^{ab}	−3	1.12 (28.28) ^{bc}	−15
30	1.30 (43.16) ^{de}	−15	1.41 (42.98) ^{bcd}	−11	0.86 (19.16) ^{de}	−31	1.00 (20.17) ^{cd}	−24
40	0.86 (25.36) ^{h}	−44	1.56 (50.21) ^{ab}	−2	0.45 (9.09) ^{hi}	−64	1.02 (20.16) ^{cd}	−23
50	0.91 (28.19) ^{gh}	−41	1.35 (38.22) ^{bcd}	−15	0.51 (10.14) ^{gh}	−59	0.75 (13.33) ^{ef}	−43
60	0.49 (11.94) ^{ij}	−68	1.18 (31.02) ^{ef}	−26	0.18 (2.60) ^{jkl}	−85	0.65 (9.72) ^{fg}	−51
70	0.33 (6.48) ^{jk}	−78	1.08 (28.63) ^{fg}	−32	0.06 (0.69) ^{klm}	−95	0.55 (8.03) ^{gh}	−59
80	0.21 (2.89) ^{kl}	−86	0.97 (21.16) ^{gh}	−39	0.02 (0.30) ^{lm}	−98	0.33 (4.41) ^{ij}	−75
90	0.09 (1.26) ^{l}	−94	0.79 (14.97) ^{h}	−50	2.41×10^{-15} (-1.14×10^{-13}) ^{m}	−100	0.21 (2.92) ^{jk}	−84
100	0.08 (0.91) ^{l}	−95	0.56 (9.87) ^{i}	−64	-4.12×10^{-15} (5.71^{-14}) ^{m}	−100	0.08 (0.74) ^{klm}	−94
p-value	0.0000				0.0000			
F-value	12.69				6.47			
$LSD_{0.05}$	0.1811				0.1650			

[x] Column means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's least significant difference. [y] Relative impact (%) = [(treatment/control) − 1] × 100. Values in brackets are untransformed means [Log (x + 1)].

3.2. Effects of *Argemone ochroleuca* Hexane Extracts on Soybean Seed Germination

The interaction between *A. ochroleuca* hexane extract concentrations and plant part were not significant ($p > 0.05$) for germination percentage, mean daily germination, germination index, plumule length and radicle length, whereas the interaction was statistically significant ($p < 0.05$) for germination speed and mean germination time. The concentration and plant parts of *A. ochroleuca* as separate factors were not significant ($p > 0.05$) for germination percentage, mean daily germination and germination index, whereas concentration as a separate factor was statistically significant ($p < 0.05$) for germination speed and mean germination time. Increasing concentrations in shoot extracts significantly decreased germination speed and mean germination time, whereas root extracts had a stimulating effect on the same variables (Table 3). Relative to untreated control, hexane shoot extract decreased germination speed (35–40%) and mean germination time (28–35%), whereas root extracts stimulated germination speed (33–53%) and mean germination time (30–35%). Concentration as a separate factor was statistically significant ($p < 0.05$) for plumule length and radicle length. Increasing concentrations in hexane extracts reduced plumule and radicle lengths by 20% and 25–27%, respectively (Table 4).

Table 3. Effect of *Argemone ochroleuca* hexane extracts on soybean germination speed and mean germination time.

Concentrations (g/L)	Germination Speed				Mean Germination Time			
	Plant Part							
	Shoots [x]	RI [y]	Roots	RI	Shoots	RI	Roots	RI
0.0	15.44 ^{ab}	-	10.81 ^{cd}	-	40.67 ^{a}	-	28.90 ^{cd}	-
2.5	15.72 ^{a}	2	16.51 ^{a}	53	39.00 ^{ab}	−4	37.50 ^{abc}	30
5.0	9.96 ^{d}	−35	14.37 ^{abc}	33	29.08 ^{bcd}	−28	39.00 ^{ab}	35
7.5	9.19 ^{d}	−40	11.48 ^{bcd}	6	26.48 ^{d}	−35	29.27 ^{bcd}	1
p-value	0.0215				0.0267			
F-value	3.60				3.40			
$LSD_{0.05}$	4.1128				9.9348			

[x] Column means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's least significant difference. [y] Relative impact (%) = [(treatment/control) − 1] × 100. Values in brackets are untransformed means [Log (x + 1)].

Table 4. Effect of *Argemone ochroleuca* hexane extracts on soybean plumule length and radicle length.

Concentrations	Plumule Length [x]	RI [y]	Radicle Length	RI
0 g/L	1.1294 ^{ab}	-	0.9209 ^{a}	-
2.5 g/L	1.2724 ^{a}	13	0.9987 ^{a}	8
5 g/L	1.0143 ^{bc}	−10	0.6685 ^{b}	−27
7.5 g/L	0.9013 ^{c}	−20	0.6928 ^{b}	−25
p-value	0.0067		0.0024	
F-value	4.12		4.86	
$LSD_{0.05}$	0.2174		0.2074	

[x] Column means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's least significant difference. [y] Relative impact (%) = [(treatment/control) − 1] × 100. Values in brackets are untransformed means [Log (x + 1)].

3.3. Effects of *Argemone ochroleuca* Acetone Extracts on Soybean Seed Germination

The interactions between *A. ochroleuca* acetone extract concentration and plant part were statistically significant ($p < 0.05$) for mean germination time and germination index,

highly significant ($p < 0.01$) for plumule length and radicle length, and not significant ($p > 0.05$) for germination percentage, germination speed and mean daily germination. Concentration as a separate factor was not significant ($p > 0.05$) for all germination variables and radicle length, whereas a significant response was observed for plumule length. Plant parts were highly significant ($p < 0.01$) for germination percentage, germination speed, mean daily germination, plumule length and radicle length, and statistically significant ($p < 0.05$) for mean germination time and germination index. Shoot and root extracts significantly decreased germination percentage, germination speed and mean daily germination by 22, 21 and 18%, respectively (Table 5). Increasing concentrations of shoot extracts did not affect the germination variables, whereas the highest concentration in the root extract significantly reduced mean germination time and germination index (Table 6). Seedling plumule and radicle were reduced by the acetone-shoot extract at the concentration of 2.5 g/L, whereas only the plumule was reduced by acetone root extract at concentrations of 7.5 g/L (Table 7). In comparison to the untreated control, shoot extracts reduced only plumule and radicle lengths by 24% and 23%, respectively, whereas *A. ochroleuca* root extracts decreased germination time, germination index, and plumule length by 16, 19 and 33%, respectively.

Table 5. Effect of *Argemone ochroleuca* acetone extracts on soybean seed germination percentage, germination speed and mean daily germination.

Plant Part	Germination Percentage [x]	RI [y]	Germination Speed	RI	Mean Daily Germination	RI
Shoots	1.27 (85.00) [a]	-	17.07 [a]	-	0.26 (0.85) [a]	-
Roots	0.99 (66.67) [b]	−22	13.43 [b]	−21	0.22 (0.67) [b]	−18
p value	0.0018		0.0076		0.0054	
F value	11.22		7.90		8.66	
$LSD_{0.5}$	0.1722		2.6137		0.0453	

[x] Column means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's least significant difference. [y] Relative impact (%) = [(treatment/control) − 1] × 100. Values in brackets are untransformed means [Arcsine (x/100)] [Log (x + 1)].

Table 6. Effect of *Argemone ochroleuca* acetone extracts on soybean mean germination time and germination index.

Concentration (g/L)	Plant Part							
	Mean Germination Time				Germination Index			
	Shoots [x]	RI [y]	Roots	RI	Shoots	RI	Roots	RI
0	1.69 (48.40) [a]	-	1.58 (37.38) [a]	-	1.03 (9.90) [a]	-	0.93 (7.70) [a]	-
2.5	1.53 (36.78) [a]	−10	1.60 (40.10) [a]	2	0.90 (7.70) [ab]	−13	0.97 (8.43) [a]	4
5	1.70 (49.25) [a]	0	1.57 (37.43) [a]	−0	1.04 (10.08) [a]	1	0.93 (7.70) [a]	−1
7.5	1.67 (46.03) [a]	−1	1.33 (25.50) [b]	−16	1.03 (9.72) [a]	−1	0.75 (5.50) [b]	−19
p value	0.0402				0.0438			
F value	3.03				2.96			
$LSD_{0.5}$	0.1958				0.1633			

[x] Column means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's least significant difference. [y] Relative impact (%) = [(treatment/control) − 1] × 100. Values in brackets are untransformed means [Log (x + 1)].

Table 7. Effects of *Argemone ochroleuca* acetone extracts on soybean plumule and radicle length (mm).

Concetrations (g/L)	Plant Part							
	Plumule				Radicle			
	Shoots [x]	RI [y]	Roots	RI	Shoots	RI	Roots	RI
0	1.71 (82.52) [ab]	-	1.36 (64.57) [c]	-	1.25 (37.82) [ab]	-	0.86 (24.98) [cd]	-
2.5	1.29 (57.45) [c]	−24	1.47 (69.78) [bc]	8	0.96 (36.13) [c]	−23	1.10 (30.92) [bc]	28
5	1.83 (106.55) [a]	7	1.38 (70.03) [c]	2	1.49 (53.33) [a]	19	0.93 (25.15) [cd]	9
7.5	1.71 (82.28) [ab]	−0	0.92 (38.55) [d]	−33	1.30 (47.45) [ab]	4	0.66 (15.62) [d]	−23
p value	0.0001				0.0005			
F value	7.31				6.00			
$LSD_{0.5}$	0.2912				0.2800			

[x] Column means followed by the same letter are not significantly different at $p \leq 0.05$ according to Fisher's least significant difference. [y] Relative impact (%) = [(treatment/control) − 1] × 100. Values in brackets are untransformed means [Log (x + 1)].

The TLC analysis was used to compare the chemical composition in the water, hexane and acetone crude extracts of *A. ochroleuca*. Similar colors with corresponding R_f values are an indication of the same chemical compounds (Tables 8 and 9). The TLC chromatograms were developed in three different solvent systems of different polarities: the CEF (intermediate polarity), EMW (polar), and BEA (non-polar) systems. The TLC chromatograms of *A. ochroleuca* shoots (S) and the root extracts (R) are shown in Figure 1. The CEF and BEA eluted more compounds as compared to EMW. Hexane extracts from shoots and roots indicated the presence of the most compounds when run with the CEF system. The observed compounds based on the color have previously been identified as flavonoids, coumarins, phenolic acids, alkaloids, saponins and anthracene derivatives. Most compounds were eluted from the acetone extracts (shoot and root) and hexane root extract in the BEA system. The fewest compounds were eluted by the EMW system. Similar compounds with an R_f value of 0.15 were identified in the shoots of water, hexane, and acetone extract while in the root, and the same compound was eluted in the acetone extract using the CEF system. A common compound with an R_f value of 0.08 was eluted in the CEF and BEA systems from the root hexane, acetone and water extract, and in the shoot hexane extract.

Table 8. R_f values of compounds separated in CEF, EMW and BEA extracted in water, hexane and acetone from the shoot of *Argemone ochroleuca*.

Solvent System	Extract	Compound Number	R_f Values of Compounds
CEF	SW	1	0.15
	SH	1	0.09
		2	0.15
		3	0.72
		4	0.85
	SA	1	0.15
		2	0.81
		3	0.86

Table 8. *Cont.*

Solvent System	Extract	Compound Number	R_f Values of Compounds
EMW	SW	1	0.07
		2	0.56
	SH	0	-
	SA	1	0.16
		2	0.62
		3	0.79
BEA	SW	0	-
	SH	1	0.08
		2	0.17
		3	0.68
	SA	1	0.08
		2	0.17
		3	0.47
		4	0.68
		5	0.94

CEF—chlorofor:ethyl acetate:formic acid; EMW—ethyl acetate:methanol:water; BEA—benzene:ethanol:ammonium hydroxide; SW—shoot water; SH—shoot hexane; SA—shoot acetone; RA—root acetone; RH—root hexane; RW—root water.

Table 9. R_f values of compounds separated in CEF, EMW and BEA extracted in water, hexane and acetone from the roots of *Argemone ochroleuca*.

Solvent System	Extract	Compound Number	R_f Values of Compounds
CEF	RA	1	0.08
		2	0.15
		3	0.61
		4	0.73
		5	0.80
		6	0.86
	RH	1	0.08
		2	0.13
		3	0.53
		4	0.62
		5	0.72
		6	0.80
		7	0.85
	RW	1	0.08
EMW	RA	1	0.12
		2	0.17
		3	0.31
		4	0.53
		5	0.63

Table 9. *Cont.*

Solvent System	Extract	Compound Number	R_f Values of Compounds
	RH	1	0.14
		2	0.19
		3	0.35
	RW	1	0.19
BEA	RA	1	0.08
		2	0.17
		3	0.23
		4	0.47
		5	0.71
	RH	1	0.08
		2	0.19
		3	0.51
		4	0.67
		5	0.75
		6	0.87
	RW	-	-

CEF—chloroform:ethyl acetate:formic acid; EMW—ethyl acetate:methanol:water; BEA—benzene:ethanol:ammonium hydroxide; SW—shoot water; SH—shoot hexane; SA—shoot acetone; RA—root acetone; RH—root hexane; RW—root water.

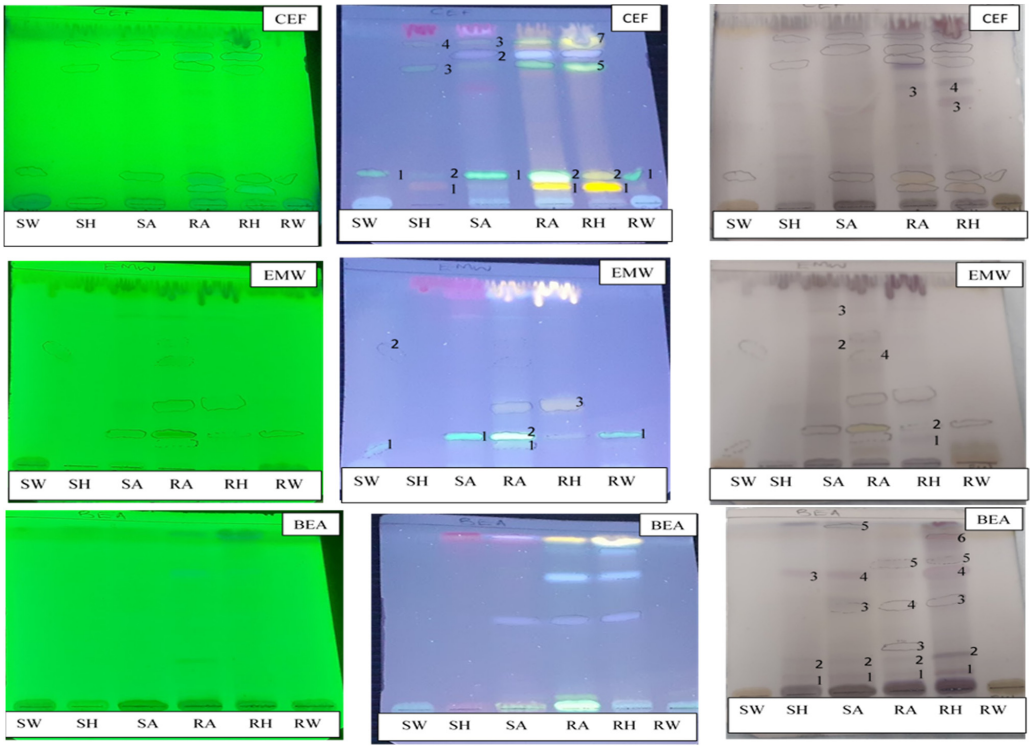

Figure 1. TLC fingerprinting of *Argemone ochroleuca* extracts, only compounds with distinct and identifiable colors are numbered. CEF—chloroform:ethyl acetate:formic acid; EMW—ethyl acetate:methanol:water; BEA—benzene:ethanol:ammonium hydroxide; SW—shoot water; SH—shoot hexane; SA—shoot acetone; RA—root acetone; RH—root hexane; RW—root water. Numbers indicate the compound number.

4. Discussion

The current study indicates that the allelopathic effects of water, hexane and acetone extracts obtained from *A. ochroleuca* shoots and roots inhibited the germination of soybean seeds, with the highest inhibition observed in the germination bioassay of water extracts. The explanation of this could be that most of the allelopathic active compound concentrations were water-soluble, hence the higher concentrations in water extracts [24]. Ashrafi et al. [24] observed that the inhibitory effects of the water-soluble fractions of *Azadirachta indica* were the highest, compared to the n-hexane-soluble and acetone-soluble fractions in all germination bioassays of *Amaranthus rotundus*, *Cirsium arvense*, *Digitaria sanguinalis*, *Sinapis arvensis*, *Lactuca sativa* and *Lolium ultiforum.* These findings were also reported by Tanveer et al. [25], who observed that there were differences in the inhibitory effects of *Euphorbia dracunculoides* n-hexane, chloroform, ethyl acetate, 1-butanol and aqueous fractions on the germination and seedling growth of maize and chickpea. Tanveer et al. [25] reported that hexane fractions had more suppressive effects on the germination of chickpeas and wheat when compared with chloroform, ethyl acetate and 1-butanol fractions. Sultana et al. [26] attributed the differences in the allelochemical composition of solvents to different compounds in plants with varying polarities and chemical properties affecting their solubility. Lower concentrations of extracts were observed to stimulate germination. This has been attributed to a process called hormesis [27]. Hormesis is an adaptative response where there is an induction of beneficial effects when the organism is exposed to low dosages of harmful chemical or physical agents [28]. In hormesis, after a small stress, special proteins responsible for the removal of damage produced by stressors are over-produced, resulting in not only the removal of damage produced by the current stress, but also the removal of the pre-existing damage, which produces a stimulating effect [29].

Weeds compete with other crops for water, nutrients and space, and they release allelochemicals into the environment that inhibit plant growth [30]. The combined effects of allelochemicals such as fatty acids, fatty acid methyl esters, terpenoids and phenolics that are released have inhibitory effects on plants [31]. Even though this particular trial did not quantify the allelochemicals, the current study has demonstrated similar decreasing trends of *A. ochroleuca* extracts' effects on the germination and seedling length of soybean seeds. These observed trends were concentration- and plant-part-dependent inhibition responses. This phenomenon is very common to many weed extracts used in crop seed germination [4,32,33]. Nxumalo et al. [33] reported a concentration-dependent inhibition response of *A. ochroleuca* extracts on the germination, seedling length and early growth of millet and maize. *Cassia occidentalis* seeds' response effects were found to be more pronounced at lower concentrations of *Psidium guajava* extracts than at higher concentrations [34]. Muche et al. [4] also reported the same effects of *A. ochroleuca* extracts on the germination and seedling length of *Sorghum bicolor* varieties. In the current study, *A. ochroleuca* extracts also affected other germination variables such as germination speed and mean daily germination. M'barek et al. [35] reported the phytotoxic effects of *Tetraclinis articulata* on germination speed, even though in their report the allelochemicals had no effects on the final germination percentage. The delay in seed germination can have some important biological and ecological implications, because it affects the ability of the seedling to establish itself in natural conditions, resulting in uneven plant stands [35].

The current study also found that there were differences in the allelopathic effects of the different plant parts of *A. ochroleuca* on the germination of soybean seeds, with extracts from the shoots inhibiting all measured germination variables more when compared to extracts from the roots. Water and acetone shoot and root extracts and hexane shoot extracts had inhibitory effects on the measured germination and seedling length variables, whereas hexane root extracts stimulated germination and seedling length variables. Generally, the distributions of allelopathic active compounds differ with plant organ, both in quantity and quality [36]. The leaves of *Eucalyptus camaldulensis* were recorded by Nasr et al. [37] to contain the highest total allelochemicals when compared to other plant organs. Ghareib et al. [38] reported that the allelopathic potential induced by low

concentrations of the acetone fraction of *Chenopodium murale* stimulated the germination and growth of tomato. According to Muche et al. [4], *A. ochroleuca* weed leaf extracts had more inhibitory effects on *Sorghum bicolor* varieties compared to extracts from the roots and stems. Root, stem and leaf aqueous extracts of *A. philoxeroides* had different effects on the root length, shoot length and fresh weight of *Z. matrella* [39]. Paul and Begum [40] explained the high degree of inhibition of the germination and seedling growth of blackgram, rapeseed and wheat using leaf and root extracts of *A. mexicana* as being due to the fact that *A. mexicana* synthesizes and stores the phytochemicals in leaves and roots.

The TLC analysis identified different classes of compounds from *A. ochroleuca* hexane and acetone extracts, which include flavonoids, lactones, phenolic acids, alkaloids, saponins and anthracene derivatives. Water extract did not elute many compounds as compared to hexane and acetone; their highly suppressive effects could mean that the compounds present in water are phytotoxic. The activities of non-polar compounds and polar ones from hexane and acetone could be attributed to the presence of the compounds shown in TLC plates. Cheng and Cheng [41] reported that the allelochemicals produced by plants exhibiting allelopathy include phenolics, terpenoids, and alkaloids, but water-soluble phenolic compounds have been established to play a major role in the growth suppression of associated plants. Phytochemicals such as phenolics, alkaloids, steroids, terpenes, saponins, and quinones have allelopathic effects on the growth and development of certain plant species [42]. According to Sasikumar et al. [43], the allelopathic effects of *Eucalyptus globulus* can be attributed to volatile terpenes and phenolic acids, and have been reported to be responsible for the inhibitory effects on the germination and seedling growth of various crops. Ghimire et al. [44] reported that alfalfa-derived phenolic compounds and saponins exhibit phytotoxicity effects on the growth of *Digitaria ciliaris*, *Chenopodium album*, *Amaranthus lividus*, *Portulaca oleracea* and *Commelina communis.* Phenolic acids have been identified and isolated from many allelopathic plants, and the role of phenolic compounds in inducing allelopathic abilities is well-established [45]. According to Movafeghi et al. [46], alkaloids, steroids, flavonoids, anthraquinones, amino acids, and polysaccharides were isolated from the seeds, leaves, flowers, stems, and roots of *Peganum harmala*, a weed reported to have phytotoxic effects on plants. Shao et al. [47] reported that alkaloids isolated from the seeds of *Peganum harmala* exerted significant inhibitory activity on lettuce, amaranth, wheat, and ryegrass seed germination. Synowiec et al. [48] reported that the essential oils of *Achillea millefolium*, *Acorus calamus*, *Carum carvi*, *Chamomilla recutita*, *Foeniculum vulgare*, *Lavandula angustifolia*, *Melissa officinalis*, *Mentha piperita*, *Salvia officinalis*, *Solidago canadensis*, *Tanacetum vulgare* and *Thymus vulgaris* inhibited the germination of *Amaranthus retroflexus*, *Avena fatua*, *Bromus secalinus* and *Centaurea cyanus*, which have been reported as notorious weeds that affect the germination of economically important crops (*Avena sativa*, *Brassica napus* and *Zea mays*). Allelopathic phytochemicals are released from donor plants as volatiles, roots exudates, or foliage leachates, and contain secondary metabolites such as flavonoid phenolics [49], ketones, aldehydes, terpenoids, lactones, cinnamic acid, and quinines [50]. When these compounds are excreted into the rhizosphere, neighboring plants absorb them through the uptake of sap [51], and interfere with the physiologic and biosynthetic machinery of the receiver plant [52,53]. Given the most recent developments in allelochemistry, which provides a physiologically and ecologically solid explanation for plant invasion, it is hypothesized that the phytotoxicity of the applied extract is responsible for the negative response of seeds or seedlings [15].

5. Conclusions

The results show that the there were differences in the allelopathic effects of the three different solvent extracts on soybean germination, with water having the highest effects. The allelopathic effects also differed between the shoot extracts and the root extracts. Lower concentrations had lesser effects on germination, while higher concentrations of *A. ochroleuca* had higher suppressive effects on germination. Flavonoids, lactones, phenolic acids, alkaloids, saponins and anthracene derivatives were observed in the extracts of *A.*

ochroleuca. The results indicate that *A. ochroleuca* has inhibitory effects on the germination of seeds, although the effect should be confirmed further by field experiments. The obtained data might be useful in the management of these weeds within soybean fields. It would be recommended for soybean producers to control this weed at the early growth stage before it causes drastic effects on the crops. This study focused on the allelopathic effects of *A. ochroleua* extracts on the germination of soybean seeds and the chemical profiling of *A. ochroleuca*; however, a similar study evaluating the effects of *A. ochroleuca* on other weeds and the modes of action of these phytochemicals would be recommended. Future research should focus on purifying, identifying, and characterizing active compounds. The obtained data would be useful in the development of bioherbicides.

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