agronomy

MDPI

Article

Phytochemistry of *Argemone ochroleuca* Sweet Extracts and Their Inhibitory Effects on Maize Seed Germination

Nezelo T. Mlombo [1,*], Zakheleni P. Dube [1], Fikile N. Makhubu [2,3] and Hellen Nxumalo [1]

[1] Faculty of Agriculture and Natural Sciences, University of Mpumalanga, Mbombela 1200, Mpumalanga, South Africa; zakheleni.dube@ump.ac.za (Z.P.D.); nxumalohellen0@gmail.com (H.N.)
[2] Faculty of Veterinary Science, University of Pretoria, Pretoria 0001, Gauteng, South Africa; makhufn@unisa.ac.za
[3] College of Agriculture and Environmental Sciences, University of South Africa, Pretoria 0001, Gauteng, South Africa
* Correspondence: nezelomlombo1@gmail.com

Abstract: *Argemone ochroleuca* Sweet is an alien invasive weed dominating most cultivated lands, however, the phytochemicals present in this plant and the effects of these on the germination and growth of economically important crops such as maize are not well-documented. The objective of the study was to characterize the phytochemistry of the shoots and roots of *A. ochroleuca* and determine whether the extracts could inhibit the germination of maize seeds. The shoots and roots of *A. ochroleuca* were extracted in water, hexane, and acetone. Ten maize seeds were used in the germination bioassay. A phytochemical analysis was conducted using gas chromatography-mass spectrometry (GC-MS). The effects of the *A. ochroleuca* water, hexane, or acetone extracts on maize seed germination were concentration and plant-part dependent. The highest reduction was recorded from the water extract with 82%. Identified compounds with high percentages in *A. ochroleuca* were 9,12-octadecadienoic acid (Z,Z) and 9,12,15-octadecatrienoic acid, (Z,Z,Z)-. The present study indicated that *A. ochroleuca* extracts suppress the germination of maize seeds, likely due to the presence of both the identified and potentially unidentified phytochemicals that were not detected by the selected method. There is, however, a need to establish the relationship between the phytochemical compounds and the enzymes responsible for germination.

Keywords: allelopathic; phytochemicals; phytotoxicity; plant extracts

check for updates

Citation: Mlombo, N.T.; Dube, Z.P.; Makhubu, F.N.; Nxumalo, H. Phytochemistry of *Argemone ochroleuca* Sweet Extracts and Their Inhibitory Effects on Maize Seed Germination. *Agronomy* **2024**, *14*, 1912. https://doi.org/10.3390/agronomy14091912

Academic Editor: Mayker Lazaro Dantas Miranda

Received: 19 July 2024
Revised: 23 August 2024
Accepted: 24 August 2024
Published: 26 August 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Maize (*Zea mays* L.) is a major crop grown all over the world and is considered the most significant grain crop [1]. Its high production is due to its several functions as a human and animal food source [2]. Maize is a staple food source for approximately 200 million people globally, contributing an estimated 15% of protein and 20% of calories to the human diet [2]. Murdia et al. [3] reported that maize can be cultivated in a variety of agro-climatic zones, which makes it an adaptable crop, in contrast to many other crops. In South Africa, it is produced throughout the country in diverse environments [4]. The top three provinces in South Africa for growing maize, which collectively contribute to nearly 83% of the country's total output, are North West, Mpumalanga, and KwaZulu-Natal [5]. South Africa [6] is ranked among the top 10 maize-producing countries in the world [6,7]. Since 1995, maize has been grown on about three to four million hectares of agricultural land, accounting for about 60 to 70% of the arable area [8]. However, the production of this crop is threatened by alien invasive weeds that release allelochemicals that inhibit seed germination and growth.

Mexican poppy [*Argemone ochroleuca* (Sweet)] is an alien invasive weed with potential biological inhibition properties to other plants [9]. Dar et al. [10] reported that both the shoot and root parts of *A. ochroleuca* released water-soluble phytotoxins that decreased

the germination of *Farsetia aegyptia Turra*, *Salvia aegyptiaca*, *Hordeum vulgare*, and *Medicago sativa*. Essential oils of *A. ochroleuca* showed significant phytotoxic activity against *Lactuca sativa* and *Peganum harmala*, thus inhibiting their germination and decreasing seedling growth [11]. These findings are supported by the study by Mlombo et al. [12] and Nxumalo et al. [13], who reported the inhibition of the germination and seedling length of common bean and maize, and millet, respectively.

A study by Paul and Begum [14] reported that the leaf and root extracts of *A. mexicana* decreased the germination of *Brassica napus*, *Triticum*, and *Vigna mungo*. Namkeleja et al. [15] reported that *A. mexicana* extract concentrations of 25, 50, 75, and 100% significantly decreased the mean germination of *Brachiaria dictyoneura* by 65, 50, 12.5, and 7.5%, respectively, and the mean germination of *Clitoria ternatea* by 77.5, 77.5, 37, and 25%, respectively. This was also observed by Miranda-Arámbula et al. [16], who reported that aqueous extracts of *A. mexicana* decreased the germination of tomato and lettuce.

Brahmachari et al. [17] reported that terpenoids, alkaloids, phenolics, and flavonoids were among the metabolites discovered through the chemical characterization of *A. mexicana.* Joshi et al. [18] also reported various kinds of secondary metabolites that included alkaloids, flavonoids, glycosides, phenol, lignins, saponins, sterols, and tannins in the phytochemical screening of *A. mexicana* leaves. The presence of allelochemicals such as phenolics and other secondary metabolites like terpenoids, and alkaloids could be responsible for inhibiting seed germination and seedling growth, and could potentially reduce the yield potential of maize. It was, however, reported that the presence and concentrations of these phytochemicals vary with the plants' growing environment [19]. Hence, the aim of this study was to characterize the phytochemistry of the shoots and roots of *A. ochroleuca* and determine whether the same extracts could inhibit the germination of maize seeds. The expected outcomes include the identification of key phytochemicals in *Argemone ochroleuca* extracts as well as evidence showing that these extracts inhibit maize seed germination.

2. Materials and Methods

2.1. Description of Study Area

The study was conducted at the University of Mpumalanga (25.4365° S, 30.9818° E), South Africa, under controlled laboratory conditions from November 2021 to May 2022. Maize seeds of cultivar SC 513 (Seed Co, Zimbabwe) were purchased from NTK, Nelspruit, South Africa.

2.2. Collection and Preparation of Argemone ochroleuca Plant Extracts

Argemone ochroleuca plant material was collected from the University of Mpumalanga farm, with the botanical identity of the plant confirmed by the H.G.W.J. Schweickerdt Herbarium (PRU), Pretoria, South Africa and given the voucher number PRU 130499. The shoots and roots of *A. ochroleuca* 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, Cape Town, South Africa) set at a temperature of 55 °C for 72 h. The dried plant material were then ground in an electric grinder (heavy duty professional blender BBS1200, Summit Pro Blend, China) and sieved into a conical flask using a 2 mm sieve.

2.3. Preparation of Crude Extracts

A total of 100 g of the shoots and roots were separately extracted in 1000 mL of water, hexane, and acetone.

Aqueous water extract: Extraction was conducted using deionized water and left at room temperature (25 °C) for 24 h. Each mixture was filtered using a 2 µm sieve, with the resultant aqueous concentrate considered as a 100% concentration. Concentrations of 10, 20, 30, 40, 50, 60, 70, 80, and 90 mL were prepared in 100 mL bottles by adding deionized water, as previously stated by Namkeleja et al. [15].

Organic solvents: A 1000 mL of acetone and hexane were separately used to extract both the shoots and roots, then the mixture was left at room temperature for 3 days with occasional stirring. The resultant filtrates were concentrated using a stream of cold air from the lamina flow to obtain a dry residue. The acetone and hexane dry root and shoot extract residues were used to make concentrations of 0, 2.5, 5, and 7.5 g/L deionized water. These dilutions were kept at room temperature for 24 h. The extracts were then centrifuged at 4000 rpm for 15 min, and the supernatant were used in the germination test [20].

2.4. Germination Bioassay

Germination tests were conducted following the techniques of the International Seed Testing Association (ISTA) [21]. Briefly, maize seeds were separately surface sterilized for 1 min by soaking them in 3% sodium hypochlorite and then washing with deionized water twice, each for 3 min. Ten seeds per treatment were set in a 9-cm diameter Petri dish lined with Whatman No. 1 filter paper. The seeds were treated with respective water extracts from the *A. ochroleuca* shoot or root concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100%, whereas for the hexane and acetone extract bioassays, the seeds were separately treated with four concentrations of 0, 2.5, 5, and 7.5 g/L. All treatments were replicated three times, and trials were duplicated three times for the water extracts and twice for the hexane and acetone extracts. The experiments were carried out under a growth chamber at room temperature 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 [15]. On the tenth day, the last germination count was made along with measurement of plumule and radicle lengths. The germination percentage, germination speed, mean germination time, mean daily germination, and germination index were computed using formulae previously described by Gairola et al. [22]:

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

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

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

$$\text{Mean germination time} = \frac{n1\ x\ d1 + n2\ x\ d2 + n3\ x\ d3 + - - - - - - - -}{Total\ number\ of\ days}$$

where n = number of germinated seed, and d = number of days.

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

$$\text{Germination index} = \frac{Number\ of\ germinated\ seeds\ on\ day\ of\ first\ count}{Day\ of\ first\ count} + \ldots \frac{Number\ of\ germinated\ seeds\ on\ day\ of\ first\ count}{Day\ of\ final\ count}$$

2.5. Phytochemical Analysis

Two grams 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 the plant material; the second collection was undertaken 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.

2.6. Phytochemical Analysis Using Gas Chromatography-Mass Spectrometry (GC-MS)

Hexane and acetone extracts from the roots and shoots of *A. ochroleuca* were analyzed using GC-MS. Gas chromatography-mass spectrometry analysis were carried out using a LECO Pegasus 4D GC-TOFMS (LECO Africa (Pty) Ltd., Kempton Park, South Africa) with a Rxi-5SilMS GC column (30 m × 0.25 mm ID × 0.2 µm film thickness) (Restek, Bellefonte, PA, USA). The following parameters were used: injection volume 1 µL; splitless injection; splitless time set at 30 s; GC inlet 250 °C; GC oven temperature program 40 °C (hold for 3 min) at 10 °C/min to 300 °C (hold for 5 min); the carrier gas used was UHP helium (Afrox, South Africa) at 1 mL/min set at constant flow mode; mass acquisition range 40–550 Da; MS transfer line temperature 280 °C; ion source temperature 230 °C; MS solvent delay 5 min; electron energy 70 eV in the electron ionization mode (EI+); data acquisition rate of 10 spectra/s; and detector voltage of 1750 V. The resulting data were combined and interpreted, and compounds corresponding to major peaks that showed a high similarity (above 85%) were recorded.

2.7. Statistical Analysis

Germination data were subjected to analysis of variance (ANOVA) through Statistix 10.0 software. The Shapiro–Wilk normality test was conducted to determine the normality of the residual distribution before ANOVA [23]. The whole number variables that failed the normality test ($p \leq 0.05$) were transformed using $\mathrm{Log}_{10}(x + 1)$, and $\mathbf{arcsine}\sqrt{(\mathbf{x} \div \mathbf{100})}$ was used for the percentage variables. Mean separation was achieved using Fisher's least significant difference test at a 5% probability level.

3. Results

There were no significant differences among the independent trials, hence the data were pooled and reanalyzed. All data for the maize seed germination variables treated with the water, acetone, and hexane extracts were not normally distributed, except for the germination speed of the hexane extract, hence the data were transformed accordingly.

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

The interaction between the water extract concentrations and plant part factors (roots and shoots) were highly significant ($p \leq 0.01$) for germination speed, mean germination time, germination index, plumule length, and radicle length, whereas the interactions were statistically significant ($p \leq 0.05$) for the germination percentage and mean daily germination. The concentration and plant parts as separate factors were highly significant ($p \leq 0.01$) for all germination variables and seedling length variables. Generally, increasing the water extract concentrations of both the root and shoot extracts significantly decreased the germination variables and seedling length variables, with significant root extract effects being observed at 70% and above for the germination variables and 50% and above for the plumule and radicle seedling lengths, whereas significant shoot extract effects were observed at 40% and above for the germination and seedling length variables (Tables 1 and 2).

In relation to the untreated control, the *A. ochroleuca* root extracts decreased the germination percentage (18–33%), germination speed (11–21%), mean germination time (13%), mean daily germination (15–29%), germination index (17%), plumule length (20–44%), and radicle length (23–52%), whereas the *A. ochroleuca* shoot extracts decreased the germination percentage (21–55%), germination speed (18–48%), mean germination time (11–32%), mean daily germination (17–62%), germination index (14–43%), plumule length (24–73%), and radicle length (32–82%), respectively. There were highly significant differences between the plant parts on all of the germination and seedling length variables, with extracts from the *A. ochroleuca* shoots significantly reducing all variables when compared to those from the roots.

Table 1. Germination response of maize seeds to *Argemone ochroleuca* water extracts.

Concentration (%)	Plant Part	Germination Percentage		Germination Speed		Mean Germination Time		Mean Daily Germination		Germination Index	
		Mean [x]	*RI* [y]	Mean	*RI*	Mean	*RI*	Mean	*RI*	Mean	*RI*
0	Roots	1.34 (92.22) [a]	-	1.22 (15.80) [a]	-	1.69 (48.40) [a]	-	0.28 (0.92) [a]	-	1.05 (10.14) [a]	-
0	Shoots	1.13 (76.67) [bcde]	-	1.12 (12.60) [abcd]	-	1.59 (39.22) [abcde]	-	0.24 (0.77) [abc]	-	0.96 (8.43) [ab]	-
10	Roots	1.24 (86.67) [abcd]	−7	1.18 (14.42) [ab]	−3	1.66 (44.92) [ab]	−2	0.27 (0.87) [ab]	−5	1.02 (9.53) [a]	−2
10	Shoots	1.27 (86.67) [abcd]	12	1.20 (14.86) [ab]	7	1.66 (45.61) [ab]	5	0.27 (0.87) [ab]	11	1.02 (9.53) [a]	6
20	Roots	1.30 (88.89) [abc]	−3	1.21 (15.18) [ab]	−1	1.67 (46.52) [a]	−1	0.28 (0.89) [ab]	−3	1.03 (9.78) [a]	−2
20	Shoots	0.94 (64.44) [efgh]	−16	1.04 (10.34) [cdefg]	−7	1.52 (33.59) [bcdef]	−4	0.21 (0.64) [cde]	−13	0.89 (7.09) [bc]	−7
30	Roots	1.36 (92.22) [a]	1	1.20 (14.94) [ab]	−2	1.69 (47.96) [a]	−0	0.28 (0.92) [a]	−0	1.05 (10.14) [a]	−0
30	Shoots	0.93 (61.11) [efgh]	−17	1.01 (9.90) [defg]	−10	1.49 (31.37) [def]	−6	0.20 (0.61) [de]	−17	0.87 (6.72) [bc]	−9
40	Roots	1.32 (92.22) [ab]	−1	1.20 (14.92) [ab]	−2	1.69 (48.34) [a]	0	0.28 (0.92) [a]	0	1.05 (10.14) [a]	0
40	Shoots	0.80 (51.11) [hij]	−30	0.91 (7.72) [gh]	−18	1.41 (26.08) [fg]	−11	0.18 (0.51) [ef]	−28	0.81 (5.62) [cd]	−16
50	Roots	1.21 (85.56) [abcd]	−10	1.16 (13.70) [abc]	−5	1.65 (44.18) [ab]	−3	0.27 (0.86) [ab]	−6	1.01 (9.41) [a]	−3
50	Shoots	0.90 (60.00) [gh]	−21	1.00 (9.45) [defg]	−10	1.49 (31.39) [cdef]	−6	0.20 (0.60) [de]	−17	0.87 (6.60) [bc]	−10
60	Roots	1.23 (85.56) [abcd]	−8	1.14 (12.96) [abc]	−7	1.65 (43.94) [abc]	−3	0.27 (0.86) [ab]	−6	1.01 (9.41) [a]	−3
60	Shoots	0.83 (54.44) [hi]	−26	0.95 (8.61) [fg]	−14	1.44 (28.22) [ef]	−9	0.19 (0.54) [e]	−24	0.82 (5.99) [c]	−14
70	Roots	1.10 (76.67) [cdef]	−18	1.09 (11.49) [bcde]	−11	1.60 (39.20) [abcd]	−6	0.25 (0.77) [abc]	−13	0.97 (8.43) [ab]	−7
70	Shoots	0.67 (38.89) [ijk]	−41	0.79 (5.74) [hi]	−29	1.28 (19.62) [gh]	−19	0.14 (0.39) [fg]	−43	0.70 (4.28) [de]	−27
80	Roots	1.08 (74.44) [defg]	−19	1.08 (11.09) [bcdef]	−12	1.58 (37.61) [abcde]	−7	0.24 (0.74) [bcd]	−15	0.96 (8.19) [ab]	−9
80	Shoots	0.62 (34.44) [jk]	−45	0.70 (4.18) [ij]	−37	1.20 (16.23) [hi]	−24	0.13 (0.34) [gh]	−48	0.65 (3.79) [ef]	−32
90	Roots	0.92 (60.00) [fgh]	−36	0.96 (8.64) [efg]	−21	1.47 (30.12) [def]	−13	0.20 (0.60) [de]	−29	0.87 (6.60) [bc]	−17
90	Shoots	0.48 (26.67) [k]	−56	0.58 (3.84) [j]	−48	0.96 (13.60) [j]	−40	0.10 (0.27) [h]	−60	0.51 (2.93) [g]	−47
100	Roots	0.90 (60.00) [gh]	−33	0.97 (8.80) [efg]	−21	1.48 (30.41) [def]	−13	0.20 (0.60) [de]	−29	0.87 (6.60) [bc]	−17
100	Shoots	0.51 (24.44) [k]	−55	0.58 (2.95) [j]	−48	1.07 (11.48) [ij]	−32	0.09 (0.24) [h]	−62	0.55 (2.69) [fg]	−43
p-value		0.0260		0.0001		0.0003		0.0013		0.0003	
F-value		2.11		3.82		3.56		3.05		3.51	
$LSD_{0.05}$		0.2043		0.1300		0.1559		0.0396		0.1121	

[x] Column means followed by the same letter were not significantly different at $p \leq 0.05$ according to Fisher's least significant difference test. [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 extract concentration on the maize plumule and radicle length (mm).

Concentrations (%)	Plumule				Radicle			
	Plant Part							
	Shoots [x]	*RI* [y]	Roots	*RI*	Shoots	*RI*	Roots	*RI*
0	1.24 (34.98) [defg]	-	1.58 (48.72) [a]	-	1.15 (32.52) [bc]	-	1.46 (41.32) [a]	-
10	1.61 (73.94) [a]	29	1.46 (46.63) [abcd]	−7	1.52 (57.11) [a]	33	1.41 (45.11) [a]	−4
20	1.22 (60.61) [efg]	−2	1.44 (42.12) [abcde]	−9	1.07 (37.89) [bcd]	−6	1.48 (47.92) [a]	1
30	1.14 (56.67) [fgh]	−9	1.54 (45.21) [ab]	−2	1.01 (39.03) [bcde]	−12	1.43 (38.09) [a]	−2
40	0.95 (46.66) [hij]	−24	1.47 (38.26) [abc]	−7	0.79 (22.67) [fg]	−32	1.42 (36.53) [a]	−3
50	1.10 (48.62) [fghi]	−11	1.27 (30.12) [cdefg]	−20	0.83 (19.16) [efg]	−28	1.13 (23.57) [bc]	−23
60	1.05 (49.26) [ghij]	−16	1.32 (33.89) [bcdef]	−16	0.82 (19.59) [efg]	−28	1.16 (22.61) [b]	−21
70	0.67 (28.59) [kl]	−46	1.19 (28.92) [fg]	−24	0.54 (12.94) [hi]	−53	0.95 (17.61) [cdef]	−35
80	0.58 (24.47) [l]	−53	1.10 (25.53) [fghi]	−30	0.44 (8.72) [ij]	−62	0.89 (15.23) [defg]	−39
90	0.48 (20.07) [lm]	−62	0.91 (20.84) [ij]	−42	0.30 (5.33) [jk]	−74	0.71 (11.50) [gh]	−52
100	0.33 (9.38) [m]	−73	0.88 (19.60) [jk]	−44	0.21 (3.62) [k]	−82	0.70 (11.21) [gh]	−52
p-value		0.0002				0.0002		
F-value		3.42				3.35		
$LSD_{0.05}$		0.2218				0.1977		

[x] Column means followed by the same letter were not significantly different at $p \leq 0.05$ according to Fisher's least significant difference test. [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 Maize Seed Germination

The interaction between the concentration and plant part of the *A. ochroleuca* hexane extract was statistically significant ($p \leq 0.05$) for the germination percentage, germination speed, mean germination time, mean daily germination, plumule length, and radicle length, whereas the interaction was not statistically significant ($p > 0.05$) for the germination index. The concentration and plant parts of *A. ochroleuca* as separate factors were not statistically significant ($p > 0.05$) for all germination variables and seedling length variables. Increasing the concentration of the shoot extracts significantly decreased the germination percentage, germination speed, mean germination time, mean daily germination, plumule length, and radicle length. At a 2.5 g/L concentration and above, the germination variables and seedling variables were reduced by the shoot extracts. The hexane root extracts had no effect on the germination variables, but stimulated the radicle length at a concentration of 7.5 g/L.

In relation to the untreated control, the *A. ochroleuca* shoot extracts decreased the germination percentage (27–38%), germination speed (31–43%), mean germination time (10–12%), mean daily germination (24–31%), plumule length (27%), and radicle length (26%), whereas the *A. ochroleuca* root extracts stimulated the radicle length by 29% (Tables 3 and 4).

Table 3. Effect of the *Argemone ochroleuca* hexane extracts on the maize germination variables.

Concentration (g/L)	Plant Part	Germination Percentage		Germination Speed		Mean Germination Time		Mean Daily Germination	
		Mean [x]	*RI* [y]	Mean	*RI*	Mean	*RI*	Mean	*RI*
0	Roots	1.04 (73.33) ^{abc}	-	12.48 ^{bc}	-	1.57 (36.57) ^{abc}	-	0.24 (0.73) ^{abc}	-
0	Shoots	1.32 (86.67) ^{a}	-	17.30 ^{a}	-	1.66 (46.73) ^{a}	-	0.27 (0.87) ^{a}	-
2.5	Roots	1.24 (83.33) ^{ab}	19	14.93 ^{ab}	20	1.64 (44.05) ^{ab}	5	0.26 (0.83) ^{ab}	10
2.5	Shoots	0.95 (61.67) ^{bc}	−27	11.99 ^{bc}	−31	1.50 (32.90) ^{bc}	−10	0.20 (0.62) ^{bc}	−24
5	Roots	1.05 (73.33) ^{abc}	1	13.18 ^{abc}	7	1.58 (38.20) ^{abc}	0	0.24 (0.73) ^{abc}	−1
5	Shoots	1.10 (75.00) ^{abc}	−16	13.55 ^{abc}	−22	1.60 (40.13) ^{abc}	−4	0.24 (0.75) ^{abc}	−10
7.5	Roots	1.14(81.67) ^{ab}	10	14.78 ^{ab}	18	1.64 (43.48) ^{ab}	5	0.26 (0.82) ^{ab}	9
7.5	Shoots	0.82 (53.33) ^{c}	−38	9.89 ^{c}	−43	1.46 (28.42) ^{c}	−12	0.18 (0.53) ^{c}	−31
p-value		0.0238		0.0300		0.0322		0.0492	
F-value		3.51		3.30		3.23		2.85	
$LSD_{0.05}$		0.3078		0.1422		0.1483		0.0591	

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

Table 4. Effect of the *Argemone ochroleuca* hexane extracts on the maize plumule and radicle lengths (mm).

Concentrations (g/L)	Plumule				Radicle			
	Plant Part							
	Shoots [x]	*RI* [y]	Roots	*RI*	Shoots	*RI*	Roots	*RI*
0	1.40 (43.35) ^{a}	-	1.22 (41.43) ^{ab}	-	1.41 (44.08) ^{a}	-	1.10 (36.93) ^{bc}	-
2.5	1.20 (60.35) ^{ab}	−15	1.36 (46.25) ^{a}	12	1.18 (59.92) ^{abc}	−16	1.34 (40.42) ^{abc}	21
5	1.37 (60.13) ^{a}	−3	1.20 (37.57) ^{ab}	−2	1.39 (63.05) ^{ab}	−1	1.26 (45.18) ^{abc}	14
7.5	1.03 (55.55) ^{b}	−27	1.37 (48.87) ^{a}	12	1.04 (53.00) ^{c}	−26	1.42 (53.92) ^{a}	29
p-value	0.0335				0.0081			
F-value	2.92				3.98			
$LSD_{0.05}$	0.2955				0.2987			

[x] Column means followed by the same letter were not significantly different at $p \leq 0.05$ according to Waller–Duncan Multiple Range test. [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 Maize Seed Germination

The interaction between the *A. ochroleuca* acetone extract concentrations and plant parts was not significant ($p > 0.05$) for all of the maize germination and seed length variables, except for the plumule length. The concentration as a separate factor was statistically significant ($p < 0.05$) for all germination variables and was highly significant ($p \leq 0.01$) for seedling length variables, whereas plant parts as a separate factor were not statistically significant ($p > 0.05$) for all germination and seedling length variables. Increasing the extract concentrations significantly decreased the germination percentage and seedling radicle length, whereas other measured variables were not different from the untreated seeds. There was a concentration-dependent response on all of the germination variables, with lower concentrations having a stimulative effect whereas higher concentrations had inhibitory effects. In relation to the untreated control, the *A. ochroleuca* extracts decreased the germination percentage (12–16%) and stimulated the radicle length by 40% (Figures 1 and 2). The acetone root extract decreased the plumule length by 25% whereas the shoot extracts stimulated the plumule length by 34% (Figure 3).

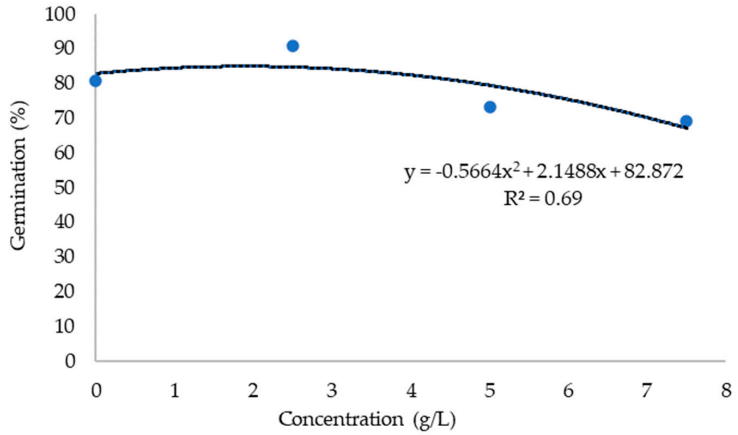

Figure 1. Phytotoxic effects of the *Argemone ochroleuca* acetone extracts on the maize germination percentage.

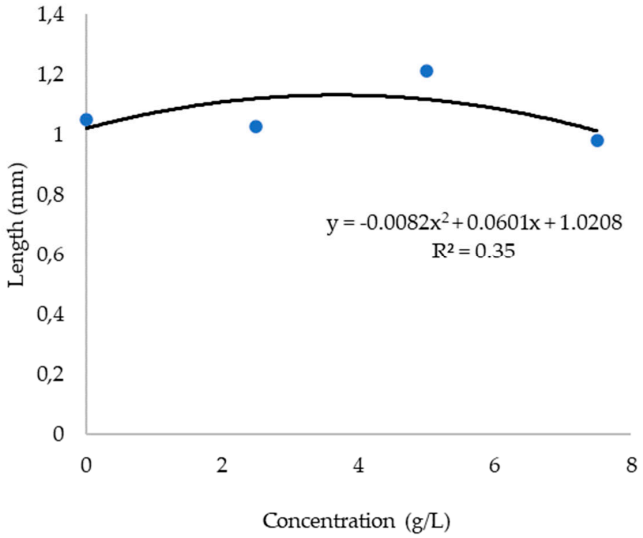

Figure 2. Phytotoxic effects of the *Argemone ochroleuca* acetone extracts on the maize radicle length.

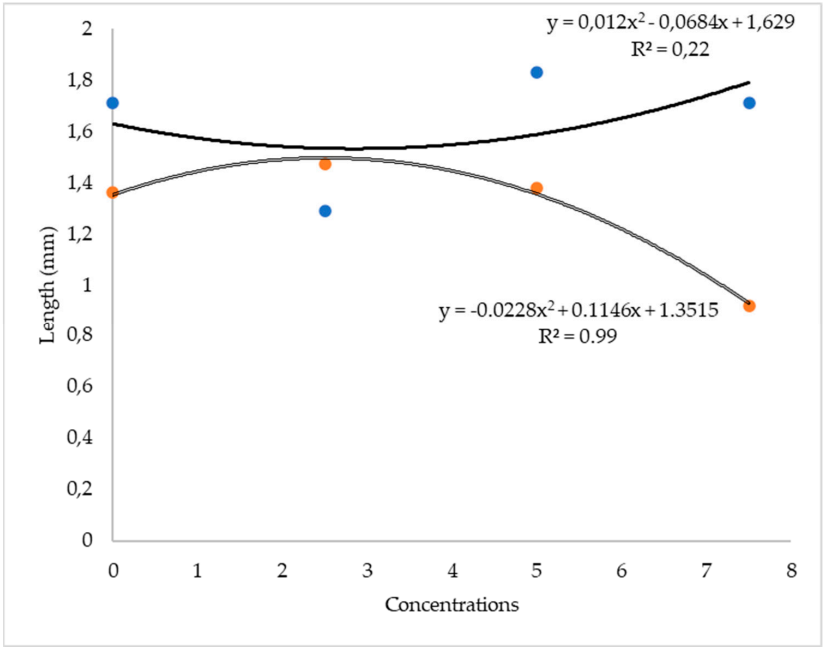

Figure 3. Effect of *Argemone ochroleuca* acetone extracts on the maize plumule length (mm).

3.4. Phytochemicals in Argemone ochroleuca

The GC-MS of the shoot and root extracts of *A. ochroleuca* identified 19 different compounds (Tables 5 and 6). The acetone extracts identified six chemical compounds from the shoots and ten from the roots, while the hexane extracts identified one from the shoots and four from the roots. Most constituents were less than 10%, and the ones that showed a high quantity were recorded. These were 9,12-octadecadienoic acid (Z,Z) followed by 9,12,15-octadecatrienoic acid, (Z,Z,Z)-, n-hexadecanoic acid, and á-sitosterol. n-Hexadecanoic acid in the shoot extracts was found to be 3%, while the same compound in the root extracts was 5%, both appearing at different retention times. 9,12-Octadecadienoic acid (Z,Z)- in the acetone shoot extracts was 2%, while in the root extracts, it was 8%, both appearing at different retention times. Hexane extracts identified nonane, 4-ethyl-5-methyl- chemical compound from the shoots and four chemical compounds in the roots. Most of the chemical constituents were less than 5%, and the ones that showed a high quantity were recorded as nonane, 4-ethyl-5-methyl-, and undecane, 2,2-dimethyl. There were no similar chemical compounds between the acetone and hexane extracts.

Table 5. Chemical profiling of the *Argemone ochroleuca* acetone shoot and root extract using GC-MS.

Name of Compound	Molecular Weight	Molecular Formula	CAS	Similarity (%)	Retention Time	Area	Plant Part
n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	57-10-3	92	9775.3	3	Shoot
Phytol	296	$C_{20}H_{40}O$	150-86-7	93	6374	1	Shoot
9,12-Octadecadienoic acid (Z,Z)-	280	$C_{18}H_{32}O_2$	60-33-3	88	1105.2	2	Shoot
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	$C_{18}H_{30}O_2$	463-40-1	89	7307.8	6	Shoot
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	292	$C_{19}H_{32}O_2$	301-00-8	86	423.97	1	Shoot
Neophytadiene	278	$C_{20}H_{38}$	504-96-1	90	912.19	1	Shoot
1-Penten-3-yne, 2-methyl-	80	C_6H_8	926-55-6	92	2437.4	1	Root
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144	$C_6H_8O_4$	28564-83-2	85	3990.6	2	Root
Hexadecenoic acid, Z-11-	254	$C_{16}H_{30}O_2$	2416-20-8	97	109.94	1	Root
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	334	$C_{20}H_{30}O_4$	85-69-8	94	44,027	1	Root
n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	57-10-3	91	12,411	5	Root
9,12-Octadecadienoic acid (Z,Z)-	280	$C_{18}H_{32}O_2$	60-33-3	92	2260.6	8	Root
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	330	$C_{19}H_{38}O_4$	23470-00-0	86	1570.3	1	Root
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	354	$C_{21}H_{38}O_4$	3443-82-1	86	1022.5	1	Root
Stigmasterol	412	$C_{29}H_{48}O$	83-48-7	88	1128.4	1	Root
á-Sitosterol	414	$C_{29}H_{50}O$	83-46-5	88	4169.5	4	Root

Table 6. Chemical profiling of the *Argemone ochroleuca* hexane shoot and root extract using GC-MS.

Name of Compound	Molecular Weight	Molecular Formula	CAS	Similarity (%)	Retention Time	Area	Plant Part
Nonane, 4-ethyl-5-methyl-	170	$C_{12}H_{26}$	1632-71-9	88	1250	3	Shoot
Undecane, 2,2-dimethyl-	184	$C_{13}H_{28}$	17312-64-0	89	503.98	3	Root
3-Pyridinecarbonitrile, 1,4-dihydro-1-methyl-	120	$C_7H_8N_2$	19424-15-8	87	2585.7	1	Root
Benzaldehyde, 3-bromo-5-methoxy-4-[(3-methylphenyl)methoxy]-	334	$C_{16}H_{15}BrO_3$	0-00-0	88	409.28	1	Root
1H-Indene, 2,3-dihydro-1,3-dimethyl-	146	$C_{11}H_{14}$	4175-53-5	85	203.74	1	Root

4. Discussion

This study showed that the *A. ochroleuca* shoots and roots have allelopathic effects that inhibit maize seed germination. Similar results have been reported in other allelopathic studies due to the inhibition of allelochemicals found in the plants [24,25]. Wang et al. [26]

reported that the enzyme activity and chlorophyll content of leaves from *Amygdalus pedunculata* varieties decreased with an increase in the aqueous leaf extract concentrations of *Amorpha fruticosa*, *Hedysarum mongolicum*, *Sabina vulgaris*, and *Hippophae rhamnoides*. Mlombo et al. [12], working with common bean seeds, observed the allelopathic effects of *A. ochroleuca* aqueous extracts on seed germination and early seedling growth. The allelopathic effects of other weed extracts such as *Argemone mexicana*, *Amaranthus hybridus*, *Parthenium hysterophorus*, and *Datura stramonium* on wheat were recorded by Gella et al. [27]. Kawawa et al. [28] explained the phenomenon as due to the allelochemicals inherent in these plants when explaining the suppressed germination and growth responses of *Cassia occidentalis* seeds to *Psidium guajava* extracts. In a study by Namkeleja et al. [15], it was observed that the *A. mexicana* leaf and seed extracts significantly decreased the seed germination, shoot length, root length, and seedling length of *Brachiaria dictyoneura* and *Clitoria ternatea.*

In the current study, it was also observed that there was a difference in the allelopathic response of the maize seed to the water, hexane, and acetone extracts. These findings were similar to findings by Akter et al. [29], who reported that aqueous extracts of *Heliotropium indicum* leaves and roots were more inhibitory compared to the methanol extracts for seed germination *Lagenaria siceraria*. Sayed et al. [30] reported that the aqueous, ethanol, and acetone extracts of honey weed significantly decreased the germination percentage of brinjal, okra, amaranth, and cucumber. These findings are concurrent with the findings from M'barek et al. [31], who reported that acetone extracts of *Tetraclinis articulate* inhibited the germination of barley, lettuce, radish, and tomato. Wang et al. [26] reported that the aqueous leaf extract concentrations of *Amorpha fruticosa*, *Hedysarum mongolicum*, and *Sabina vulgaris* at lower concentrations significantly promoted the seed germination and seedling growth of two *Amygdalus pedunculata* varieties. This is in agreement with the findings in the current study where lower extract concentrations stimulated the germination and germination variables, with the effect much more prominent in the hexane root extracts. This may be attributed to the stress effect of these extracts on plants, and may stimulate plants to defend themselves by increasing the concentration of some metabolites such as amino acids, proteins, phenolics, and carbohydrates, a phenomenon known as hormesis [26]. The concentration-dependent responses of the plants to the water, hexane, and acetone fractions revealed that all three fractions contained allelochemicals, but that the greatest potential was in the water-soluble fraction [32]. The method used in extraction has also been reported by other studies to influence the extract yield, hence the resultant allelochemical composition of the extract [33].

There was a difference in the allelopathic effects of the different plant parts whereby the shoot extracts were inhibitory when compared to the root extracts. It was also reported in a study by Paul and Begum [14] that the leaf and root extracts of *Argemone mexicana* had different suppressive effects on the germination of wheat, blackgram, and rapeseed, whereas in some cases, the root extracts were more suppressive, and in others, the leaf extracts had more suppressive effects. This was also observed by Huang et al. [34], who reported that lower concentrations of *Alternanthera philoxeroides* root extracts stimulated the root length, shoot length, and fresh weight of *Zoysia matrella,* while higher concentrations inhibited the variables. This was also reported by Sitthinoi et al. [35], who observed that different extraction solvents and plant parts from where the extract was made showed differences in the inhibitory effect on the germination and seedling growth of rice. These findings conform to a study conducted by Netsere [36], who reported that there were differences in the allelopathic response of different plant parts of *Parthenium hysterophorus* on the germination and seedling growth of maize and sorghum. Similar findings were reported by Sitthinoi et al. [35], who reported that the shoot extracts from jungle rice showed higher inhibitory effects on the seedling dry weight and root length of rice compared to the root extracts. Favaretto et al. [37] explained that the consistence of leaves as a source of the allelochemicals in various studies could not only be explained by their role as storage organs, but also that they are the most metabolically active plant organs in the plant body, hence it is reasonable that they have a greater diversity of allelochemicals and thus a greater

allelopathic effect. Based on the results from the current study, it is evident that *A. ochroleuca* contains allelochemicals that affect the germination, plumule, and radicle length of maize.

Profiled chemicals in the roots and shoots indicated various chemicals including fatty acids, sterols, and terpenoides. Previous studies have reported that the *Argemone* species contain phytochemicals such as benzoic acid, coptisine, cinnamic, protopine, berberine [38,39], and phenolic compounds such as salicylic acid, vanillic acid, and p-hydroxy benzoic acid [40]. These chemicals have been reported to potentially inhibit the germination and seedling vigor of crops [9]. Tahir et al. [25] reported that moringa extracts were rich in octadec-9-enoic acid, nonacosane, methyl 12,15-octadecadienoate and 9-octadecenoic acid, and methyl ester, (E)-; in the same study, it was reported that these extracts inhibited and stimulated the germination and seedling growth of wheat and *Sinapus arvensis*.

The presence of 9,12,15-octadecatrienoic acid, (Z,Z,Z)-, n-hexadecanoic acid, octadecadienoic acid (Z,Z)-, and á-sitosterol in the extracts of *A. ochroleuca* with their allelopathic effects on seed germination in the present study is supported by several studies. Wang et al. [41] reported similar compounds as the current study, which were extracted and identified from *Humulus scandens* root extracts and had potential allelopathic effects on *Alternanthera philoxeroides*. Tahir et al. [25] also reported that 9,12-octadecadienoic acid (Z,Z), identified from *Pistacia atlantica* Kurdica hexane leaf and seed extracts, was a possible allelochemical that caused inhibition in the germination of *Triticum aestivum* and *Sinapis arvensis* seeds. 9,12-Octadecadienoic acid (Z,Z)- was identified as an effective allelochemical that had a significant allelopathic effect on weed suppression [42]. Tahir et al. [25] further reported that 9,12-octadecadienoic acid was significantly correlated with the inhibition in the germination of *Triticum aestivum* and *Sinapis arvensis* seeds. 9,12,15-Octadecatrienoic acid (Z,Z,Z) was extracted from *Carduus nutans* and n-hexadecanoic acid was extracted from *Cirsium creticum* and *Carduus nutans* and identified as some of the chemicals that had an allelopathic effect on the germination of *Raphanus sativus*, *Lactuca sativa*, and *Lepidium sativum* [43]. According to studies conducted by Zhang et al. [44] and Yuan et al. [45], the identified n-hexadecanoic acid in the *Solidago canadensis* and *Spartina alterniflora* aqueous extracts had significant allelopathic effects on the growth of wheat and algae. Zhang et al. [44] also noted that the higher the n-hexadecanoic acid concentration, the greater the inhibitory effect on the shoot and root lengths of wheat. Verma et al. [42] identified 9,12,15-octadecatrienoic acid, methyl ester, and phytol from sunnhemp, 9,12-octadecadienoic acid (Z,Z)- from sesame and neophytadiene, and 9,12-octadecadienoic acid (Z,Z) from pearl millet, which were effective as allelochemicals when used in weed control. The presence of different allelochemicals may be responsible for the inhibitory effects of various extracts on the germination and seedling growth of the *Microcystis aeruginosa* blooms [41]. Several phenolic compounds present in *A. ochroleuca* are known to be biologically active, and they could also be potential allelochemicals [25,42]. Since more than one compound with allelopathic potential was discovered in this plant in the present study, it could be speculated that synergistic effects of these potential allelochemicals could exist for this plant to exert its potential allelopathic effects on other plants [46]. As allelopathic substances, these chemical compounds should be further investigated as well as their effects on the germination of seeds. Some of these chemical compounds have been identified and used in pharmaceutical studies and in the development of bioherbicides [47,48].

5. Conclusions

The current study details the allelopathic effects of the *A. ochroleuca* shoot and root water, hexane, and acetone extracts on the germination of maize seeds and the phytochemicals present in this plant. The allelopathic effects of the three distinct extracts on maize differed from one another. Additionally, the allelopathic effects varied between the two plant parts, with the shoot extracts having higher inhibitive effects than the root extracts. There was a concentration-dependent response in maize seed germination, while *A. ochroleuca* at higher concentrations had drastic effects on germination, and lower concentrations had

less of an effect. The presence of 9,12,15-octadecatrienoic acid, (Z,Z,Z)-, n-hexadecanoic acid, octadecadienoic acid (Z,Z)-, á-sitosterol, and other compounds in the extracts of *A. ochroleuca* could have allelopathic effects on seed germination, as shown in the present study. The extracted phytochemicals might have phytotoxic effects on other weeds, which could be exploited and used alternatively as environmentally friendly herbicides. *Argemone ochroleuca* is an invasive alien weed with allelopathic effects on other plants. This weed releases phytochemicals into the environment that inhibit the germination and growth of other crops in the vicinity, and it is for this reason that this weed should be controlled at an early stage before it affects the production of crops. This study was conducted in a laboratory, meaning that the controlled conditions may not entirely duplicate naturally in field settings, thus resulting in variances in the germination rates. Furthermore, the choice of solvent and analytical technique may result in insufficient extraction and characterization of the phytochemicals, reducing the comprehensiveness of the phytochemical profile. Future research should consider conducting similar studies in the field to better understand the ecological relevance of the findings as well as using a broader range of solvents and other analytical techniques to provide a more comprehensive profile of the plant's phytochemicals.

Author Contributions: Conceptualization, N.T.M., Z.P.D. and F.N.M.; Methodology, N.T.M., Z.P.D., F.N.M. and H.N.; Validation, N.T.M., Z.P.D. and F.N.M.; Formal analysis, N.T.M., Z.P.D., F.N.M. and H.N.; Investigation, N.T.M., Z.P.D., F.N.M. and H.N.; Resources, N.T.M., Z.P.D. and F.N.M.; Data curation, N.T.M., Z.P.D., F.N.M. and H.N.; Writing—original draft preparation, N.T.M., Z.P.D. and F.N.M.; Writing—review and editing, N.T.M., Z.P.D. and F.N.M.; Visualization N.T.M., Z.P.D. and F.N.M.; Supervision, Z.P.D. and F.N.M.; Sourcing funding, N.T.M. and Z.P.D.; Project administration, N.T.M., Z.P.D., F.N.M. and H.N. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Mpumalanga (Vice Chancellor Scholarship) 201743337 and the Nation Research Foundation (NRF) MND210617612476.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: The authors duly acknowledge the H.G.W.J. Schweickerdt Herbarium (PRU) at the University of Pretoria.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Yadava, P.; Abhishek, A.; Singh, R.; Singh, I.; Kaul, T.; Pattanayak, A.; Agrawal, P.K. Advances in maize transformation technologies and development of transgenic maize. *Front. Plant Sci.* **2017**, *7*, 19–49. [CrossRef] [PubMed]
2. Nuss, E.T.; Tanumihardjo, S.A. Maize: A paramount staple crop in the context of global nutrition. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 417–436. [CrossRef] [PubMed]
3. Murdia, L.K.; Wadhwani, R.; Wadhawan, N.; Bajpai, P.; Shekhawat, S. Maize utilization in India: An overview. *Am. J. Food Nutr.* **2016**, *4*, 169–176.
4. Fanadzo, M.; Chiduza, C.; Mnkeni, P.N.S. Effect of inter-row spacing and plant population on weed dynamics and maize (*Zea mays* L.) yield at Zanyokwe irrigation scheme, Eastern Cape, South Africa. *Afr. J. Agric. Res.* **2010**, *5*, 518–523.
5. Adisa, O.M.; Botai, J.O.; Adeola, A.M.; Hassen, A.; Botai, C.M.; Darkey, D.; Tesfamariam, E. Application of artificial neural network for predicting maize production in South Africa. *Sustainability* **2019**, *11*, 1145. [CrossRef]
6. Gouse, M.; Pray, C.; Schimmelpfennig, D.; Kirsten, J. Three seasons of subsistence insect-resistant maize in South Africa: Have smallholders benefited. *AgBioForum* **2006**, *9*, 15–22.
7. Food and Agricultural Organization of the United Nations (FAO). The State of Food Insecurity in the World 2008: High Food Prices and Food Security-Threats and Opportunities. 2008. Available online: http://www.fao.org/docrep/011/i0291e/i0291e00.htm (accessed on 3 May 2021).
8. Moshia, M.E.; Newete, S.W. Mexican poppy (*Argemone mexicana*) control in cornfield using deep learning neural networks: A perspective. *Acta Agric. Scand. B Soil Plant Sci.* **2018**, *69*, 1–7. [CrossRef]
9. Muche, M.; Molla, E.; Teshome, H. Phytotoxicity of *Argemone ochroleuca* L. on germination and seedling growth of *Sorghum bicolor* L. varieties under in vitro condition. *Am. Eurasian J. Agric. Environ. Sci.* **2018**, *18*, 185–192.

10. Dar, B.A.; Al-Rowaily, S.L.; Assaeed, A.M.; El-Bana, M.I.; Hegazy, A.K.; Malik, J.A. Allelopathic potential of *Argemone ochroleuca* from different habitats on seed germination of native species and cultivated crops. *Pak. J. Bot.* **2017**, *49*, 1841–1848.
11. Abd-ElGawad, A.M.; El Gendy, A.E.N.G.; Assaeed, A.M.; Al-Rowaily, S.L.; Omer, E.A.; Dar, B.A.; Al-Taisan, W.A.A.; Elshamy, A.I. Essential oil enriched with oxygenated constituents from invasive plant *Argemone ochroleuca* exhibited potent phytotoxic effects. *Plants* **2020**, *9*, 998. [CrossRef]
12. Mlombo, N.; Dube, Z.P.; Ganyani, L.; Nxumalo, H.; Mnyambo, N.M.; Timana, M. *Argemone ochrolueca* extracts suppression of germination and early growth of bean (*Phaseolus vulgaris*). *Res. Crops* **2021**, *22*, 508–515.
13. Nxumalo, H.; Dube, Z.P.; Ganyani, L.; Mlombo, N.T.; Timana, M.; Mnyambo, N.M. Potential suppressive effects of Mexican poppy weed residues on germination and early growth of maize and pearl millet crops. *Afr. J. Food Agric. Nutr. Dev.* **2022**, *22*, 19909–19928. [CrossRef]
14. Paul, N.K.; Begum, N. Influence of root and leaf extracts of *Argemone mexicana* on germination and seedling growth of blackgram, rapeseed and wheat. *Bangladesh J. Sci. Ind. Res.* **2007**, *42*, 229–234. [CrossRef]
15. Namkeleja, H.S.; Tarimo, M.T.; Ndakidemi, P.A. Allelopathic effect of aqueous extract of *Argemone mexicana* L on germination and growth of *Brachiaria dictyoneura* L. and *Clitoria ternatea* L. *Am. J. Plant Sci.* **2013**, *4*, 2138–2147. [CrossRef]
16. Miranda-Arámbula, M.; Reyes-Chilpa, R.; Anaya, A.L. Phytotoxic activity of aqueous extracts of ruderal plants and its potential application to tomato crop. *Bot. Sci.* **2021**, *99*, 487–498. [CrossRef]
17. Brahmachari, G.; Gorai, D.; Roy, R. *Argemone mexicana*: Chemical and pharmacological aspects. *Rev. Bras. Farmacogn.* **2013**, *23*, 559–567. [CrossRef]
18. Joshi, N.; Bhatt, S.; Dhyani, S.; Nain, J. Phytochemical screening of secondary metabolites of *Argemone mexicana* linn. Flowers. *Int. J. Curr. Pharm. Res.* **2013**, *5*, 144–147.
19. Namkeleja, H.S.; Tarimo, M.T.C.; Ndakidemi, P.A. Allelopathic effects of *Argemone mexicana* to growth of native plant species. *Am. J. Plant Sci.* **2014**, *5*, 1336–1344. [CrossRef]
20. Tarzi, B.G.; Gharachorloo, M.; Baharinia, M.; Mortazavi, S.A. The effect of germination on phenolic content and antioxidant activity of chickpea. *Iran. J. Pharm. Sci.* **2012**, *11*, 1137–1143.
21. Jones, S. Future seed testing needs for seed analysts and researchers-A personal view. *Seed Technol.* **2003**, *35*, 15–20.
22. Gairola, K.C.; Nautiyal, A.R.; Dwivedi, A.K. Effect of temperatures and germination media on seed germination of *Jatropha curcas* Linn. *Adv. Biores.* **2011**, *2*, 66–71.
23. Gomez, A.; Gomez, A.A. *Statistical Procedures for Agricultural Research*, 2nd ed.; John Wiley and Sons: New York, NY, USA, 1984.
24. Rodríguez-Caballero, G.; Caravaca, F.; Alguacil, M.M.; Fernández-López, M.; Fernández González, A.J.; Roldán, A. Striking alterations in the soil bacterial community structure and functioning of the biological N cycle induced by *Pennisetum setaceum* invasion in a semiarid environment. *Soil Biol. Biochem.* **2017**, *109*, 176–187. [CrossRef]
25. Tahir, N.A.; Qader, K.O.; Azeez, H.A.; Rashid, J.S. Inhibitory allelopathic effects of *Moringa oleifera* Lamk plant extracts on wheat and *Sinapis arvensis* L. *Allelopath. J.* **2018**, *44*, 35–48. [CrossRef]
26. Wang, X.; Wang, J.; Zhang, R.; Huang, Y.; Feng, S.; Ma, X.; Zhang, Y.; Sikdar, A.; Roy, R. Allelopathic effects of aqueous leaf extracts from four shrub species on seed germination and initial growth of *Amygdalus pedunculata* Pall. *Forests* **2018**, *9*, 711. [CrossRef]
27. Gella, D.; Ashagre, H.; Negewo, T. Allelopathic effect of aqueous extracts of major weed species plant parts on germination and growth of wheat. *J. Agric. Crop Res.* **2013**, *1*, 30–35.
28. Kawawa, R.C.A.; Muyekho, F.N.; Obiri, J.F.; Agevi, H.; Obiet, L. The allelopathic impact of *Psidium guajava* L., leaf extracts on the germination and growth of *Cassia occidentalis* L., seeds. *J. Agric. Vet. Sci.* **2016**, *9*, 101–105.
29. Akter, P.; Siddiqua, T.; Begum, R.; Ahmed, A.A. Evaluation of the allelopathic activity of aqueous and methanol extracts of *Heliotropium indicum* leaves and roots on eight Cucurbit crops. *Horticulturae* **2024**, *10*, 135. [CrossRef]
30. Sayed, M.A.; Haque, M.M.; Roy, B.; Hossain, S.M.J.; Das, S.R. Allelopathic effects of different extracts of honeyweed (*Leonurus siribicus*) on seeds germination and seedlings growth of some selected vegetables. *J. Nat. Prod.* **2012**, *5*, 243–250.
31. M'barek, K.; Zribi, I.; Haouala, R. Allelopathic effects of *Tetraclinis articulata* on barley, lettuce, radish and tomato. *Allelopath. J.* **2018**, *43*, 187–202. [CrossRef]
32. Ashrafi, Z.Y.; Sadeghi, S.; Alizade, H.M.; Mashhadi, H.R.; Mohamadi, E.R. Study of bioassay the allelopathical effect of Neem (*Azadirachta indica*) n-hexane, acetone and water-soluble extracts on six weeds. *Int. J. Biol.* **2009**, *1*, 71–77. [CrossRef]
33. Sultana, B.; Anwar, F.; Ashraf, M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules* **2009**, *14*, 2167–2180. [CrossRef] [PubMed]
34. Huang, Y.; Ge, Y.; Wang, Q.; Zhou, H.; Liu, W.; Christie, P. Allelopathic effects of aqueous extracts of *Alternanthera philoxeroides* on the growth of *Zoysia matrella*. *Pol. J. Environ. Stud.* **2017**, *26*, 97–105. [CrossRef] [PubMed]
35. Sitthinoi, P.; Lertmongkol, S.; Chanprasert, W.; Vajrodaya, S. Allelopathic effects of jungle rice (*Echinochloa colona* (L.) Link) extract on seed germination and seedling growth of rice. *Agric. Nat. Resour.* **2017**, *51*, 74–78. [CrossRef]
36. Netsere, A. Allelopathic effects of aqueous extracts of an invasive alien weed *Parthenium hysterophorus* L. on maize and sorghum seed germination and seedling growth. *J. Biol. Agric. Health Sci.* **2015**, *5*, 120–124.
37. Favaretto, A.; Chini, S.; Scheffer-basso, S.; Sobottka, A.; Bertol, C.; Perez, N. Pattern of allelochemical distribution in leaves and roots of tough lovegrass (*Eragrostis plana* Nees.). *Aust. J. Crop Sci.* **2015**, *9*, 1119–1125.
38. Facchini, P.J. Alkaloid biosynthesis in plants: Biochemistry, cell biology, molecular regulation and metabolic engineering applications. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2001**, *52*, 9–66. [CrossRef]

39. Cheng, F.; Cheng, Z. Research progress on the use of plant allelopathy in agriculture and physiological and ecological mechanisms of allelopathy. *Front. Plant Sci.* **2015**, *6*, 10–20. [CrossRef]
40. Burhan, N.; Shaukat, S.S. Allelopathic potential of *Argemone Mexicana* L. A tropical weed. *Pak. J. Biol. Sci.* **1999**, *2*, 1268–1273. [CrossRef]
41. Wang, L.; Liu, Y.; Zhu, X.; Zhang, Z.; Huang, X. Identify potential allelochemicals from *Humulus scandens* (Lour.) Merr. root extracts that induce allelopathy on *Alternanthera philoxeroides* (Mart.) Griseb. *Sci. Rep.* **2021**, *11*, 1–8.
42. Verma, P.; Blaise, D.; Sheeba, J.A.; Manikandan, A. Allelopathic potential and allelochemicals in different intercrops for weed management in rainfed cotton. *Curr. Sci.* **2021**, *120*, 1035–1039. [CrossRef]
43. Formisano, C.; Rigano, D.; Senatore, F.; Feo, V.D.; Bruno, M.; Rosselli, S. Composition and allelopathic effect of essential oils of two thistles: *Cirsium creticum* (Lam.) D.U'rv. ssp. triumfetti (Lacaita) Werner and *Carduus nutans* L. *J. Plant Interact.* **2007**, *2*, 115–120. [CrossRef]
44. Zhang, D.; Ye, Y.; Li, J.; Dong, L. Allelopathic pathways, isolation and identification of an allelopathic substance from *Solidago canadensis* L. *Allelopath. J.* **2014**, *33*, 201–212.
45. Yuan, R.; Li, Y.; Li, J.; Ji, S.; Wang, S.; Kong, F. The allelopathic effects of aqueous extracts from *Spartina alterniflora* on controlling the *Microcystis aeruginosa* blooms. *Sci. Total Environ.* **2020**, *712*, 136332. [CrossRef] [PubMed]
46. Yan, J.; Bi, H.H.; Liu, Y.Z.; Zhang, M.; Zhou, Z.Y.; Tan, J.W. Phenolic compounds from *Merremia umbellata* subsp. orientalis and their allelopathic effects on Arabidopsis seed germination. *Molecules* **2010**, *15*, 8241–8250. [CrossRef] [PubMed]
47. Sheela, D.; Uthayakumari, F. GC-MS analysis of bioactive constituents from coastal sand dune taxon—*Sesuvium portulacastrum* (L.). *Biosci. Discov.* **2013**, *4*, 47–53.
48. Sweetlin, P.; Daniel, R.R. Determination of bioactive compounds in ethanolic extract of callus derived from *Mucuna pruriens* using gas chromatography and mass spectroscopic technique. *J. Nat. Remedies* **2020**, *21*, 11–16.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.