



Allelopathic Effects of Aqueous Extracts from Select Weed Species on the Germination Rate and Seedling Development of Fenugreek and Associated Weeds

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Abstract

A laboratory experiment was conducted at the Department of Field Crops laboratory at the College of Agriculture, University of Tikrit, to evaluate the allelopathic influence of the aqueous extracts from some weed species on the germination and early growth of fenugreek and its companion weeds that growth with it in fields. The trial was conducted following a Completely Randomized Design (CRD) utilizing independent contrasts, and the study consisted of 12 experimental units so each containing 10 seeds per tested species namely *Sonchus oleraceus*. (annual sowthistle), *Eruca sativa* (arugula, garden rocket), and *Trigonella foenum-graecum* (fenugreek). These seeds were treated individually one by one with various aqueous extracts such as *Eucalyptus* extract, *Silybum marianum* (milk thistle) extract and arugula extract and a distilled water for control treatment. Phenotypic traits evaluated encompassed the germination percentage of weed seeds and plumule along with radicle lengths as well as the dry weight of both plumule and radicle for both fenugreek and the associated weeds.

The results appear that the *Eucalyptus* extract exerted a significant inhibitory effect on *Sonchus oleraceus* seeds, considerably a reduce of the germination percentage also plumule length and radicle length, with mean values recording 10%, 2.833 cm, and 1.667 cm, respectively. Similarly, when *Eruca sativa* seeds were treated with *Eucalyptus* extract. the lowest mean values for germination percentage, plumule length, and radicle length were observed, yielding 43.30%, 3.533 cm, and 2.133 cm, respectively. Conversely, regarding the germination and seedling growth of *Trigonella foenum-graecum*, the *Silybum marianum* extract revealed a positive stimulatory effect achieving the highest means at 96.67%, 9.033 cm and 7.377 cm respectively. concerning the dry weight of the plumule and radicle for the fenugreek plant, the *Silybum marianum* extract treatment recorded the maximum mean values, reaching 0.0900 g and 0.03533 g respectively.

Regarding the dry weight of the weeds, the *Eucalyptus* extract significantly suppressed biomass accumulation, recording the lowest dry weight means for *Sonchus oleraceus*.at 0.003667 g and for *Eruca sativa* at 0.01467 g.

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1. Introduction

Fenugreek (*Trigonella foenum-graecum*) is an essential leguminous and medicinal crop. however, its productivity is severely constrained by intense weed competition. This interference extends beyond classical competition for essential growth resources such as water, nutrients, light, and space to encompass complex allelopathic interactions. Weeds non-stop synthesize and exude bio-toxic metabolites into the surrounding environment, which can severely prevent seed germination patterns or disturbing

subsequent embryonic development. Fenugreek is known as one of the oldest medicinal and nutritive plants that belongs to the Fabaceae family. It is widely cultivated across diverse regions for its multifaceted uses in traditional ethnopharmacology also in modern pharmaceutical industries and food development (Petropoulos, 2002)^[39]. For Economic and medical purposes, the high value of fenugreek seeds comes from their important biochemical profile which features bioactive compounds like saponins, alkaloids also flavonoids and soluble dietary fibers (Mehrafarin *et al.*, 2011)^[34]. Fenugreek seeds generally have a high germination capacity and their physiological performance remains highly vulnerable to surrounding ecosystem stressors and chemical exudates released by nearby vegetation, it is a biochemical phenomenon known as allelopathy (Alsaadawi *et al.*, 2012)^[5]. Therefore, evaluating the bio-inhibitory effects of botanical extracts on the germination dynamics and early development of fenugreek is critical and sensitive to deciphering these interspecific chemical interactions within field ecosystems. Leading by these dynamics, recent agricultural research has increasingly focused and aimed on investigating plant-derived allelochemicals as ecologically sustainable as bio-herbicide alternatives to synthetic agrochemicals for weed management (Alsaadawi *et al.*, 2012)^[5]. Weed plant species have a competitive faculty that enables them to inhibit or completely displace nearby (associated) crop plants. This ecology dominance is achieved by either through the aggressive depletion of essential nutrients or by the exuding of phytotoxic chemical compounds that actively inhibit surrounding vegetation growth (FAO, 2012)^[17]. Also these invasive species are characterized by their prolific seed production and the clear unique capacity for precocious seed germination while still attached to the maternal plant, a reproductive strategy prominently noticed in annual sowthistle (*Sonchus oleraceus*). (Al-Hassawi & Al-Joubouri, 1989)^[1]. Many scientific studies have highlighted the physiological sensitivity of fenugreek to allelopathic interactions. For example, Alsaadawi *et al.* (2012)^[5] mentioned that a 10% aqueous extract derived from river red gum (*Eucalyptus camaldulensis*) leaves induced a severe and statistically significant reduction in fenugreek seed germination, leading to a 68% decline relative to the control treatment. So as for Al-Khazaali (2018)^[3] emphasized that the biological potency of these botanical extracts is severely concentration-dependent, while elevated concentrations exert severe bio-toxic suppression, lower thresholds can start a stimulatory response. Allelopathy is an example for a pivotal ecological mechanism for sustainable weed management, utilized through cropping systems largely and rotational strategies by applying aqueous extracts from crop residues to suppress (inhibit) weed growth (Farooq *et al.*, 2011)^[18]. Therefore, the encompassed term refers to biological processes involving the synthesis and release of secondary metabolites not only from plants but also from microorganisms such as bacteria, viruses, and fungi which collectively dictate seed germination, and vegetative growth, also macro-biological system dynamics (Narwal & Sampietro, 2009)^[37]. For a complex ecological phenomenon, the allelopathy involves

multifaceted biochemical interactions mediated by the releasing of specialized secondary compounds known as allelochemicals, entering the surrounding biophysical environment by diverted pathways, and exerting either phytotoxic (inhibitory) or hormetic (stimulatory) influences on the physiological functions of recipient organisms such as crops (Lahmood, 2012). These metabolites can be made from both living tissues and decomposing plant matter, normally arising as secondary configurations whose precise primary metabolic pathways remain undefined or known (Kamal, 2011)^[28]. Basically, there is a difference between allelopathy and resource competition. While competition entails the aggressive acquisition of essential resources from the shared environment, allelopathy works through the active addition of bioactive chemical materials into the biosphere (Rice, 1984)^[40]. The activity of this phenomenon is dependent on the chronological age of the donor plant, and as for the concentration and chemical profile of released compounds. The research indicates that as donor growing plants advance in maturity, the concentration of built-up allelochemicals within various plant organs increases proportionally. This physiological shift intensifies the detrimental effects on seed germination so as yield components and growth traits of recipient species (Al-Rawi, 2001; Al-Juheishi, 2005; Saleh, 2008b)^[4]. Ecosystems in field is completely isolating the individual effects of resource competition from allelopathic biochemical inhibition remains continuing challenge, as failures in germination and later yield dynamics are continuously confounded by ambient microflora and fluctuating environmental variables (Bezuidenhout, 2008)^[9]. To know this complexity, Muller (1969)^[36] introduced the comprehensive term interference, bridging both allelopathy and resource competition as a defined cumulative net interactions exerted between neighboring plant species (as cited in Finney, 2004)^[20]. Allelopathy is biological alternative among many suggested ways approaches capturing the attention of agricultural researchers looking to innovate sustainable weed control and management frameworks and promote crop productivity (Chou, 1999; Hozayn *et al.*, 2011)^[10, 23]. A literature explains that multiple strategies can be deployed to utilize allelopathic concepts for weed suppression. One of these primarily include the foliar application of aqueous extracts derived from highly phytotoxic plant species, or can be the direct incorporation of their tissue residues into the soil matrix to restrict weed proliferation and subsequently improve crop growth dynamics and yield architecture. Several researchers have called for the use of these water-soluble extracts as a friendly ecosystem as bio-herbicides. These formulations can be directly integrated into integrated weed management practices or forms as natural templates for developing study, structurally safer and ecologically alternatives of benign crop-protection (Jamil, 2004)^[27].

2. Materials and Methods.

An experimental study was conducted for evaluating the potential of allelopathic in aqueous extracts from specific plant species on the germination dynamics and early seedling growth development of fenugreek (*Trigonella foenum-*

graecum) and its associated weeds. The laboratory study was carried out at the Department of Field Crops- College of Agriculture, University of Tikrit.

2.1. Preparation of Aqueous Extracts.

Aqueous extracts were made up at a uniform concentration of 2% (w/v). exactly by 20 g of dried plant residues from Eucalyptus (*Eucalyptus spp.*), milk thistle (*Silybum marianum*) and mallow (*Malva sylvestris*) were separately blended one by one with 1 liter of distilled water following the extraction protocol which was described by Mersie and Singh (1987a).

The resultant mixture of distilled water and plant powder was homogenized by using an electric blender for 15 minutes. For purification, each crude sample was initially filtered through three layers of medical gauze (cheesecloth) to remove coarse debris, followed by fine filtration using Whatman No. 1 filter papers to yield a clear extract. The final filtrate for each respective plant species was transferred into tightly sealed, opaque glass containers and stored in a refrigerator at 5°C to maintain stability until subsequent experimental use (El-Khawas & Shehata, 2005).

2.2. Cultivation Protocol and Bioassay Procedure.

To evaluate the allelopathic influence of the prepared aqueous extracts on germination and phenotypic growth of fenugreek and its associated weeds. laboratory bioassays were conducted utilizing disposable Petri dishes with a diameter of 13.8 cm. Each Petri dish was lined with two layers of Whatman No. 1 filter papers. Ten uniform seeds of each respective plant species which was, fenugreek (*Trigonella foenum-graecum*), annual sowthistle (*Sonchus oleraceus*) and garden rocket (*Eruca sativa*) were introduced independently into the dishes. Each experimental treatment was established in triplicate.

Subsequently a fixed volume of 8 mL of either the designated plant extract or distilled water (which served as the control treatment) was applied to the respective Petri dishes. All treated and control dishes were then transferred into a Seed Germinator (Agrosaw) maintained at a constant incubation temperature of 25°C.

The germination percentage was determined after an incubation period of eight days. Upon the conclusion of a 14 day experimental duration. the developed seedlings were carefully harvested and dissected to separate the radicle from the plumule. Then the lengths of radicles and plumules were recorded. To determine the biomass accumulation, the separated seedling components were dried by using an electric oven at 70°C for 72 hours until a constant weight was maintain, after that their dry weights were precisely measured (Saleh, 2009).

2.3. Experimental Treatments.

The laboratory study had 12 distinct treatment combinations, structured as follows:

1: Aqueous extract of Eucalyptus spp. applied to *Sonchus oleraceus* (annual sowthistle).

- 2: Aqueous extract of *Silybum marianum* applied to *Sonchus oleraceus* (annual sowthistle).
- 3: Aqueous extract of *Malva sylvestris* applied to *Sonchus oleraceus* (annual sowthistle).
- 4: Control treatment (distilled water) applied to *Sonchus oleraceus* (annual sowthistle).
- 5: Aqueous extract of *Eucalyptus spp.* applied to *Eruca sativa* (Garden rocket).
- 6: Aqueous extract of *Silybum marianum* applied to *Eruca sativa* (Garden rocket).
- 7: Aqueous extract of *Malva sylvestris* applied to *Eruca sativa* (Garden rocket).
- 8: Control treatment (distilled water) applied to *Eruca sativa* (Garden rocket).
- 9: Aqueous extract of *Eucalyptus spp.* applied to *Trigonella foenum-graecum* (Fenugreek).
- 10: Aqueous extract of *Silybum marianum* applied to *Trigonella foenum-graecum* (Fenugreek).
- 11: Aqueous extract of *Malva sylvestris* applied to *Trigonella foenum-graecum* (Fenugreek).
- 12: Control treatment (distilled water) applied to *Trigonella foenum-graecum* (Fenugreek).

2.4. Laboratory Measurements and Parameters.

2.4.1. Germination Percentage (GP %).

The final germination percentage was determined for each experimental treatment eight days post-sowing. The percentage was calculated using the following standard physiological equation by (Saied, 1984) ^[41].

Germination Percentage (GP%) = (Total number of germinated seeds \ Total number of planted seeds) * 100.

2.4.2. Plumule Length (cm).

Fourteen days post sowing five seedlings were randomly selected from each Petri dish. The length of the embryonic shoot (plumule) was measured from the point of seed emergence to the apex of the shoot (Saleh, 2009) ^[42].

2.4.3. Radicle Length (cm).

Utilizing the same randomly selected seedlings, the length of the radicle was determined by measuring from its point of emergence from the seed coat to its terminal root tip (Ali, 1982) ^[2].

2.4.4. Plumule Dry Weight (mg).

The embryonic shoots (plumules) from the sampled seedlings were excised and subjected to thermal drying utilizing an electric oven maintained at 70°C for a continuous duration of 72 hours. after dehydration, the dry biomass was precisely quantified using a high-precision analytical balance (ISTA, 2012) ^[26].

2.4.5. Radicle Dry Weight (mg).

The excised radicles were collected and were dried by using an oven under exact conditions (70°C for 72 hours). The final dry weight of the radicle biomass was recorded using a high-precision analytical scale (ISTA, 2012) ^[26].

2.4.6. Statistical Analysis.

collected data from this laboratory experiment study were statistically analyzed as a factorial experiment carried out under a Completely Randomized Design (CRD). The analysis of variance (ANOVA) was used utilizing the Statistical Analysis System (SAS) software package. Duncan's Multiple Range Test was used to compare and evaluate treatment means at a significance level of 0.05.

3. Results and Discussion.

3.1. Germination Percentage (GP %).

The statistical analysis in table 1 revealed significant variations (0.05) among the experimental treatments regarding the seed germination percentage, the aqueous extracts of Eucalyptus spp., Silybum marianum, and Malva sylvestris exhibited a pronounced inhibitory effect on the germination dynamics of the targeted weeds (Sonchus oleraceus and Eruca sativa). On the other hand, no statistically significant reduction was noticed in the germination of fenugreek (*Trigonella foenum-graecum*) seeds.

These outcomes show the differential allelopathic potential of these plants' extracts, clearing of their efficacy in suppressing weed growth and their possibilities as natural sources for bio-herbicidal compounds. also, the results refer to the magnitude of this phytotoxic inhibition depends fundamentally on both the specific weed species (the recipient and the donor plant) residue utilized. This specificity reveals the critical importance of evaluating targeted extract-weed interactions.

As for the application of Eucalyptus spp. extract to Sonchus oleraceus and Eruca sativa seeds resulted in the lowest seed germination percentages which recorded 10% and 43.30%, respectively. This severe phytotoxicity may be attributed to the rich presence of phenolic compounds and volatile essential oils, such as 1,8-cineole, in eucalyptus tissues. These allelochemicals are mentioned to disrupt mitotic cellular division and induce severe lipid peroxidation, may leading to the degradation of cell membrane integrity (Singh *et al.*, 2020) [43].

also, the same eucalyptus extract exerted no detrimental effect on fenugreek germination. however, Sonchus oleraceus recorded the highest susceptibility to all three plant extracts. Meanwhile, fenugreek seeds show a remarkable allelopathic tolerance, with the germination percentage remaining notably high at 96.67%, even when exposed to a high toxic eucalyptus extract. This resilience in fenugreek may be fundamentally ascribed to the physical thickness and structural density of its seed coat, coupled with an efficient endogenous antioxidant defense system that neutralizes allelochemical-induced oxidative stress.

3.2. Plumule Length (cm).

The statistical analysis recorded in Table 1 indicates high significant differences among the experimental treatments regarding plumule elongation across the studied plant. The findings show a pronounced phytotoxic suppression of plumule growth in the targeted weeds using the application of the botanical extracts.

The application of Eucalyptus spp. extract to Sonchus oleraceus and Eruca sativa seeds causing severe growth retardation, resulted the shortest plumule lengths of 2.83 cm and 3.55 cm, respectively. This effect of inhibition may be governed by the high concentration of bioactive allelochemicals embedded within eucalyptus foliar tissues, most notably volatile monoterpenes such as 1,8-cineole and pinene alongside key phenolic acids including ferulic and caffeic acids. These results align with Singh *et al.* (2020) [43], who reported a 78% reduction in mustard plumule length he used a 10% aqueous eucalyptus extract. also, the documentation by El-Khatib *et al.* (2022) [14], which classified Sonchus oleraceus as one of the most susceptible weeds to eucalyptus-mediated allelopathy.

When Silybum marianum was applied to fenugreek seeds, a distinct stimulatory phenomenon was observed, making the maximum plumule length of 9.03 cm. This growth improvement may be explained by the concept of hormesis, which is a biphasic dose response mechanism wherein exposure to sub-lethal or low concentrations of phytotoxic agents triggers an adaptive stimulatory response in resilient organisms. As For as a hard crop like fenugreek, the used extract concentration may serve as a mild, non-lethal stressor (eustress) that upregulates the biosynthesis of endogenous growth-promoting phytohormones, like gibberellins as a defensive countermeasure while concurrently activating antioxidant enzyme systems. also, Silybum marianum may characterized by its high content of silymarin, a potent flavonolignan complex known to act as an effective antioxidant and growth-stimulant at micro-dose thresholds.

This treatment was affected by the Eucalyptus spp. extract (7.84 cm) and Malva sylvestris extract (7.63 cm). While these botanical treatments did not exhibit statistically significant variations among the treatments, they all mathematically surpassed the untreated control treatment (7.20 cm). These noticed patterns are highly consistent with the studies of Duke (2015) [11], who said that sub-toxic thresholds of phenolic compounds can enhance legume development by 15% to 30%.

3.3. Radicle Length (cm).

The experimental results recorded in Table 1 shows a clear reduction in radicle length of both Sonchus oleraceus and Eruca sativa seedlings following exposure to the aqueous tissue extracts of Eucalyptus spp. also Silybum marianum, and Malva sylvestris.

A comparative evaluation of the treatment means show that the extracts of Silybum marianum, Malva sylvestris, and Eucalyptus spp. severely suppressed radicle growth development in Sonchus oleraceus, by giving minimal average values of 1.27 cm, 1.38 cm, and 1.67 cm, respectively. Similarly, Eruca sativa seeds gave a significant Flaw when treated with Eucalyptus spp. and Malva sylvestris extracts, which registered the lowest radicle length averages at 2.13 cm and 2.57 cm, respectively.

This ability noticed in the radicles of both weed species is biologically attributed to the fact that the radicle is the primordial embryonic structure to emerge during germination. So that it maintains direct physical contact with

the phytotoxic solution, making it sensitive to allelopathic interference exceptionally.

Regarding the cellular level this inhibition maybe led by the structural destruction of the root cap cells. Volatile monoterpenes present in eucalyptus, such as 1,8-cineole, possess lipophilic properties that emulsify the lipid bilayer of the cell membranes within the root cap, activating cellular necrosis. With no functional root cap, the radicle loses its gravitropic perception and mechanical ability to penetrate growth media (Li *et al.*, 2019) [31].

These findings are highly consistent with Oueslati (2003) [38],

Table 1: The Allelopathic effects of extracts from select weed species on the germination percentage, plumule length, and radicle length of fenugreek (*Trigonella foenum-graecum*) and its associated weeds.

No.	Treatment Details (Extract + Target Plant)	Germination Percentage (GP %)	Plumule Length (cm)	Radicle Length (cm)
1	Eucalyptus spp. aqueous extract applied to <i>Sonchus oleraceus</i> seeds (Sun spurge).	10.0 f	2.833 f	1.667 hi
2	<i>Silybum marianum</i> aqueous extract applied to <i>Sonchus oleraceus</i> seeds (Sun spurge).	60.0 c	4.050 de	1.267 i
3	<i>Malva sylvestris</i> aqueous extract applied to <i>Sonchus oleraceus</i> seeds (Sun spurge).	73.33 b	3.200 ef	1.377 i
4	Control treatment (distilled water) applied to <i>Sonchus oleraceus</i> seeds (Sun spurge).	100 a	3.933 de	1.967 gh
5	Eucalyptus spp. aqueous extract applied to <i>Eruca sativa</i> seeds (Garden rocket).	43.30 e	3.533 e	2.133 fg
6	<i>Silybum marianum</i> aqueous extract applied to <i>Eruca sativa</i> seeds (Garden rocket).	53.33 d	6.443 c	3.277 e
7	<i>Malva sylvestris</i> aqueous extract applied to <i>Eruca sativa</i> seeds (Garden rocket).	63.33 c	4.500 d	2.567 f
8	Control treatment (distilled water) applied to <i>Eruca sativa</i> seeds (Garden rocket).	100 a	4.533 d	4.067 d
9	Eucalyptus spp. aqueous extract applied to <i>Trigonella foenum-graecum</i> seeds (Fenugreek).	96.67 a	7.843 b	4.590 c
10	<i>Silybum marianum</i> aqueous extract applied to <i>Trigonella foenum-graecum</i> seeds (Fenugreek).	96.67 a	9.033 a	7.377 a
11	<i>Malva sylvestris</i> aqueous extract applied to <i>Trigonella foenum-graecum</i> seeds (Fenugreek).	96.67 a	7.633 b	5.533 b
12	Control treatment (distilled water) applied to <i>Trigonella foenum-graecum</i> seeds (Fenugreek).	100 a	7.200 b	4.700 c

*Treatments followed by the same letter do not differ significantly at the 5% probability.

3.4. Plumule Dry Weight (g).

The statistical data registered in Table 2 show a significant difference among the aqueous extract treatments about the plumule dry weight of fenugreek. The application of *Silybum marianum* extract exhibited a distinct and significant superiority by yielding the maximum dry biomass accumulation with a mean of 0.090 g.

These results are agreed with Farooq *et al.* (2020) [19], who mentioned that priming fenugreek seeds with *Silybum marianum* extract improved the dry weight of the vegetative shoot system by 38% compared to the control treatment. They also conformed the study by El-Darier and Youssef (2018) [13], which verified that specific flavonoids can rise up biomass accumulation in leguminous plants by 25% to 40%. The *Malva sylvestris* extract treatment ranked second, recording a plumule dry weight of 0.080 g, followed by the untreated control treatment, which registered 0.053 g.

Vice versa, the lowest average plumule dry weight was registered under the *Eucalyptus* spp. extract treatment, falling to 0.047 g. This reduction of biomass induced by the

who declared a reduction by 90% in lettuce radicle length upon exposure to a 10% eucalyptus extract. They also agree with Hussain *et al.* (2020) [24], who mentioned that *Silybum marianum* extracts inhibit radicle elongation in Asteraceae weeds by up to 88%. On the other hand, a contrasting response was registered in the cash crop, where the *Silybum marianum* extract was applied to fenugreek seeds yielded the maximum radicle length reaching an average of 7.38 cm.

eucalyptus aqueous extract maybe driven by the presence of 1,8-cineole and associated monoterpenes. These phytotoxic compounds may speed up chlorophyll degradation by systematically disrupting the structural integrity of both chlorophyll a and b molecules. Consequently, this degradation severely impairs photosynthetic efficiency and limits the biosynthesis of essential soluble carbohydrates, as studied and documented by Batish *et al.* (2008) [8].

also, these results agree with Zhang *et al.* (2019) [44], who documented that eucalyptus extracts reduced wheat plumule dry weight by 25%, despite a paradoxical 15% increase in plumule length. This metabolic inhibition can be supported by Zhang and Fu (2010) [45], who confirmed that 1,8-cineole exposure triggers a 30% reduction in total endogenous protein content within developing seedlings.

3.5. Radicle Dry Weight (g).

The results in Table 2 demonstrate a significant difference among the aqueous extract treatments regarding the radicle dry weight of fenugreek. Interestingly, all applied weed

extracts induced a statistically significant increase in radicle dry biomass compared to the control treatment.

Obtaining the maximum dry weights under the *Silybum marianum* and *Malva sylvestris* extract treatments factor, which registered 0.035 g and 0.034 g, respectively, exhibiting no mutually significant variations. Also, these were followed by the *Eucalyptus* spp. extract treatment, which registered a dry weight for the radicle of 0.018 g, while the untreated control treatment yielded the lowest value of 0.008 g.

This substantial biomass accumulation maybe started by both *Silybum marianum* and *Malva sylvestris* extracts is biophysically attributed to the phenomenon of hormesis. Under these sub toxic chemical thresholds, active ingredients such as silymarin and various flavonoids operate as biological stimulants. These compounds empower epidermal cell division, induce lateral root ramification and upregulate nutrient assimilation efficiency (Grossmann, 2010; Khan *et al.*, 2021) [22, 30].

Also, silymarin could improve the enzymatic activity of Phenylalanine Ammonia-Lyase, a key enzyme responsible for driving the phenylpropanoid pathway and the subsequent deposition of lignin within root tissues, so that it can make

increasing overall radicle dry mass. These findings align closely with Al-Watban and Salama (2021) [7], who mentioned a 4.2-fold increase in the radicle dry weight of fenugreek when treated with *Silybum marianum* extract.

The statistically significant biomass increase recorded in the *Eucalyptus* spp. extract treatment despite its documented phytotoxicity to the vegetative shoot system possibly explained as an adaptive defense mechanism. This stress-induced response involves the accelerated deposition of lignin and cellulose within the cell walls of the radicle as a countermeasure forcing the chemical stress exerted by 1,8-cineole. Lignin, acting as a highly structural and dense dry substance, directly enhances the cumulative dry mass of the radicle (Lyu & Blum, 1990) [32]. In addition, specific monoterpenes showed to stimulate the initiation of adventitious roots as a compensatory physiological mechanism to remove the structural damage sustained by the root apical meristem (Singh *et al.*, 2020) [43]. This result is supported by Singh *et al.* (2020) [43], who mentioned a paradoxical increase by 80% in the dry weight of mustard roots under eucalyptus allelopathic stress.

Table 2: The Allelopathic effects of extracts from select weed species on the plumule dry weight and radicle dry weight of fenugreek (*Trigonella foenum-graecum*) seedlings.

No.	Treatment Details (Extract + Target Plant)	Plumule dry Wight (g)	Radicle dry Wight (g)
1	<i>Eucalyptus</i> spp. aqueous extract applied to <i>Trigonella foenum-graecum</i> seeds (Fenugreek).	0.04733 d	0.01833 b
2	<i>Silybum marianum</i> aqueous extract applied to <i>Trigonella foenum-graecum</i> seeds (Fenugreek).	0.09000 a	0.03533 a
3	<i>Malva sylvestris</i> aqueous extract applied to <i>Trigonella foenum-graecum</i> seeds (Fenugreek).	0.07967 b	0.03467 a
4	Control treatment (distilled water) applied to <i>Trigonella foenum-graecum</i> seeds (Fenugreek).	0.05333 c	0.00800 c

*Treatments followed by the same letter do not differ significantly at the 5% probability.

3.6. Dry Weight of annual sowthistle (*Sonchus oleraceus*) (g).

The experimental data registered in Table 3 show a significant inhibitory effect of all evaluated aqueous extracts on the seedling dry weight of *Sonchus oleraceus*. The untreated treatment recorded the maximum dry biomass accumulation, by average of 0.0093 g. but, the *Eucalyptus* spp. extract exerted the most potent phytotoxic suppression, significantly reducing the seedling dry weight to a minimum of mean (0.0037 g). The extracts of *Malva sylvestris* and *Silybum marianum* yielded intermediate dry weight values of 0.0053 g and 0.0057 g respectively.

This severe biomass restriction maybe because of the exceptional susceptibility of *Sonchus oleraceus* to botanical allelochemicals which aligns with its classification within vulnerable families sensitive to competitive biochemical stress (Khaliq *et al.*, 2020) [29]. At the physiological level, 1,8-cineole present in eucalyptus tissues maybe accelerates the degradation of chlorophyll molecules and directly inhibits the

enzymatic activity of Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco). This dual mechanism can drastically lower photosynthetic efficiency and impairs the subsequent accumulation of dry matter (Batish *et al.*, 2008; Singh *et al.*, 2020) [8, 43].

Also, maybe the water-soluble phenolic compounds characterized in *Silybum marianum* and *Malva sylvestris* extracts actively suppress the nitrate reductase enzyme system. This enzymatic inhibition disrupts nitrogen assimilation, thereby stopping crucial protein biosynthesis within susceptible recipient species (Einheilig, 1995) [12]. These results correspond closely with El-Khatib *et al.* (2022) [14], who documented a 65% reduction in the dry weight of *Sonchus oleraceus* subjected to eucalyptus allelopathic stress. They further corroborate the studies of Hussain *et al.* (2020) [24], which confirmed that *Silybum marianum* tissue extracts can prevent the total biomass accumulation of sensitive weed species by 40% to 50%.

Table 3: The Allelopathic effects of extracts from select weed species on the seedling dry weight of annual sowthistle (*Sonchus oleraceus*).

No.	Treatment Details (Extract + Target Plant)	Dry Wight (g)
1	Eucalyptus spp. aqueous extract applied to <i>Sonchus oleraceus</i> seeds (Sun spurge).	0.003667 c
2	<i>Silybum marianum</i> aqueous extract applied to <i>Sonchus oleraceus</i> seeds (Sun spurge).	0.005670 b
3	<i>Malva sylvestris</i> aqueous extract applied to <i>Sonchus oleraceus</i> seeds (Sun spurge).	0.005333 b
4	Control treatment (distilled water) applied to <i>Sonchus oleraceus</i> seeds (Sun spurge).	0.009333 a

*Treatments followed by the same letter do not differ significantly at the 5% probability.

3.7. Seedling Dry Weight of Garden Rocket (*Eruca sativa*) (g)

The experimental results presented in Table 4 reveal significant differences for the aqueous extract treatments regarding the seedling dry weight of *Eruca sativa*. The treatment of *Silybum marianum* and *Malva sylvestris* extracts exhibited high superiority, by giving the maximum dry biomass accumulation with averages of 0.0243 g and 0.0247 g, respectively, with no demonstrating of any mutually significant variations. The *Eucalyptus* spp. extract treatment recorded a dry weight of 0.0147 g, while the control treatment registered the lowest mean value of 0.0103 g.

This statistically significant increase in the dry weight of *Eruca sativa* seedlings across all botanical extract treatments maybe because of the attributed to the high endogenous resilience characteristic of Brassicaceae species against competitive allelopathic chemicals, along with the biophysical mechanism of hormesis. Under these sub-toxic chemical thresholds, the active components function effectively as biological growth stimulants (Duke, 2015) [11]. Also, the pronounced superiority of the *Silybum marianum* and *Malva sylvestris* treatments could be driven by their rich profiles of water-soluble phenolic and flavonoid compounds, most notably quercetin, kaempferol, and silymarin complexes. These bio-compounds actively can regulate the enzymatic activity of Phenylalanine Ammonia Lyase, which leads the downstream phenylpropanoid pathway and subsequent structural lignin deposition. Concurrently, they improve of the nitrate reductase enzyme system, which speed up nitrate reduction and essential protein biosynthesis, thereby maximizing dry matter accumulation within the seedling tissues (Ghorbanpour *et al.*, 2021; Khan *et al.*, 2021) [21, 30].

These results are highly consistent with Mahmoud *et al.* (2020) [33], who recorded a 125% increase in the seedling dry weight of *Eruca sativa* following treatment with *Silybum marianum* extract. They further concur with Al-Sherif (2022) [6], who confirmed that flavonoids can enhance the total biomass accumulation of Brassicaceae species by up to 140%.

On the other hand, the significant stimulatory effect observed under the *Eucalyptus* spp. extract treatment—despite the presence of the phytotoxic 1,8-cineole—is biophysically explained by the activation of the endogenous glucosinolate–myrosinase defense system inherent to garden rocket. Within this metabolic pathway, the myrosinase enzyme hydrolyzes glucosinolates into isothiocyanates, which function effectively as biological growth promoters at sub-lethal concentrations. Concurrently, these bioactive derivatives stimulate lateral root proliferation and accelerate cell wall lignification as an adaptive response to mild chemical stress (eustress) (Fahey *et al.*, 2001; Lyu & Blum, 1990) [16, 32]. These observation patterns align closely with Zhang *et al.* (2019) [44], who documented a 40% increase in the dry weight of radish (*Raphanus sativus*) seedlings subjected to eucalyptus allelopathic stress.

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Table 4: The Allelopathic effects of extracts from select weed species on the seedling dry weight of garden rocket (*Eruca sativa*).

No.	Treatment Details (Extract + Target Plant)	Dry Wight (g)
1	<i>Eucalyptus</i> spp. aqueous extract applied to <i>Eruca sativa</i> seeds (Garden rocket).	0.01467 b
2	<i>Silybum marianum</i> aqueous extract applied to <i>Eruca sativa</i> seeds (Garden rocket).	0.02433 a
3	<i>Malva sylvestris</i> aqueous extract applied to <i>Eruca sativa</i> seeds (Garden rocket).	0.02467 a
4	Control treatment (distilled water) applied to <i>Eruca sativa</i> seeds (Garden rocket).	0.01033 c

*Treatments followed by the same letter do not differ significantly at the 5% probability

4. Conclusions

Laboratory experiment study reveals a distinct species-specific phytotoxic influence, showing that allelopathic efficacy depends on donor residue-recipient plant interaction. *Eucalyptus spp.* extracts demonstrated an effected herbicidal treatment against weeds, while an exceptional tolerance showed by *Trigonella foenum-graecum* and *Silybum marianum* induced positive hermetic stimulation.

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