



International Journal of Agriculture Natural Farming Research

The Allelopathic Effect of Some Aqueous Extracts of Eucalyptus, Milk Thistle, And Mallow on The Germination and Growth of Basil, Arugula, And Sow Thistle Seeds

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Article Info

E-ISSN: 3107-7145

Volume: 02

Issue: 03

Received: 23-04-2026

Accepted: 25-05-2026

Published: 27-06-2026

Page No: 10-14

Abstract

This laboratory experiment was conducted in the labs of the Department of Field Crops at the College of Agriculture, University of Tikrit. The study aimed to investigate the allelopathic effect of some aqueous extracts of Eucalyptus (*Eucalyptus* spp.), Milk thistle (*Silybum marianum*) and Mallow (*Malva sylvestris*) on the germination and growth of Basil (*Ocimum basilicum* L.), Arugula (*Eruca sativa*) and common Sow thistle (*Sonchus oleraceus*) seeds. The experiment was applied using a completely randomized design (independent comparisons). The trial included 12 experimental units and each unit contained 10 seeds for each type of used seeds (Basil, Arugula, and Sow thistle). These seeds were treated with the aqueous extracts individually. The extracts used in this experiment were Eucalyptus plant extract, Milk thistle weed extract, and mallow weed extract, in addition to the control treatment without adding any extract. The studied traits included the germination percentage of the weed seeds, radicle and plumule length also the dry weight of the radicle and plumule for Basil and its associated weeds.

The results showed that the Eucalyptus extract led to the inhibition of the germination percentage for Sow thistle and Arugula seeds with means of 46.67 and 53.30%, respectively. Regarding the plumule length we notice that the mallow extract gave the lowest value for Sow thistle and Arugula plumule length by reaching 2.00 and 2.833 cm, respectively, while the effect of Eucalyptus extract on the plumule length of Arugula reached 2.177 cm.

The results also showed that the mallow extract affected the radicle length of Sow thistle and Arugula and their means were 0.467 and 1.433 cm respectively. The Eucalyptus extract had a clear effect on the radicle length feature for Sow thistle and Arugula where the length for them was 1.220 and 1.267 cm, respectively.

On the other hand, the dry weight of the plumule of Basil plant, the Milk thistle extract treatment recorded its highest mean which was 0.0233 g. Also the Milk thistle extract recorded the highest mean for the dry weight trait of Sow thistle and Arugula by reaching a mean of 0.02133 and 0.0390 g, respectively.

DOI: <https://doi.org/10.54660/IJANFR.2026.2.3.10-14>

Keywords: Allelopathic effect, Basil, Weeds, Sow thistle, Extract

1. Introduction

Basil (*Ocimum basilicum* L.) is considered one of the most important medicinal and aromatic plants belonging to the Lamiaceae family. It is characterized by containing important volatile oils like linalool, methyl chavicol, and eugenol which are used in the pharmaceutical, food, and cosmetic industries. Basil is widely cultivated in Iraq and the Arab region due to its high economic and medicinal value and as its leaves and seeds are used as a digestive stimulant, antibacterial, and antioxidant (Simon *et al.*,

1990; Makri & Kintzios, 2008) ^[10, 7].

The stage of germination and early growth is one of the most critical stages in the life of the Basil plant, as it is greatly affected by the surrounding environmental conditions especially competition with weed plants which are defined as unwanted plants in the same place as the crop where they compete with the crop for water, light, nutrients, and space. Also some weeds have allelopathic effects, meaning they secrete secondary chemical compounds that negatively affect the germination and growth of the accompanied crop (Zimdahl, 2007; Rice, 1984) ^[14, 9].

Many studies have proved that the presence of weeds in the early growth stages of Basil leads to a significant decrease in the germination percentage, plumule and radicle length also the dry weight of the plant. as a result of direct competition and the allelopathic effect. Therefore, there was a need to study the allelopathic effect of plant extracts such as Eucalyptus and Milk thistle along with mallow as alternative bio-herbicides to chemical pesticides that cause environmental pollution (El-Khawas, S.A. and Shehata, M.M., 2005; Dayan, F.E., Cantrell, C.L. and Duke, S.O., 2009) ^[6, 4].

Among the most knowing weeds that are found with Basil and medicinal crops are Sow thistle (*Sonchus oleraceus*) and wild Arugula (*Eruca sativa*). Sow thistle weed is characterized by a deep and widespread root system and its high growth rate which makes the competition very strong and it also secretes phenolic compounds that have inhibitory effects on the germination of many crops. As for Arugula weed it is considered one of the fast-germinating plants and has a short life cycle that consumes large amounts of nitrogen and reduces the growth of Basil (Qasem *et al.*, 2001; Anjum, 2005) ^[8, 2].

Weeds are one of the most difficult problems facing the agricultural area locally and globally, as the percentage of losses caused by weeds in crop fields ranges from (45 - 90%) of the economic yield. This is due to the type of crop and environmental conditions, and the effects of weeds are not limited to competition only, but it was found that they have the ability to secrete chemicals that lead to the prevention or obstruction of germination and growth of neighboring plants, which is known as allelopathic substances, as is the case with many other plants, and it is estimated that there are more than ten thousand chemical substances secreted from different parts of them.

The phenomenon of allelopathy is defined as the direct or indirect effect of a chemical compound secreted by a plant on another plant through root secretions, washing of plant leaves or the decomposition of plant remains. These compounds play an important role in determining the type and composition of the vegetation cover in the ecosystem (Rice, 1984) ^[9].

The allelopathy phenomenon is considered one of the most important alternatives that researchers have focused on for weed management and improving crop growth (Chou, 1999; Hozayn *et al.*, 2011).

There are many studies proved that the aqueous extracts of some plants like Eucalyptus and Mallow contain phenolic compounds such as flavonoids and terpenes that cause significant inhibition in the germination percentage of Basil seeds and radicle also plumule length. This inhibition increases with the increase of extract concentration (Al-Snafi, 2016; Tawaha & Turk, 2003) ^[1, 13]. The reason for this inhibition is may due to the effect of allelopathic compounds

on physiological processes inside the seed, such as water absorption, cell division enzymes activity, and the respiration process (Einhellig, 1996) ^[5].

The allelopathy phenomenon differs from the concept of competition, where competition is defined as taking materials from the environment while allelopathy happens when adding chemical materials to the environment that affect other plants and organisms (Rice, 1984) ^[9]. It differs according to the age of the plants and the concentration and type of these materials. Also some research proved that with the advance of plant age the number of stored materials in its different parts may increase and therefore the negative effect on germination, yield components and growth parameters of the receiving plant increases (Al-Rawi, 2001; Al-Jahaishi, 2005; Saleh, 2008b).

Studies show in many occasion that there are many strategies through which we can use the concept of allelopathy in weed control and limiting their spread. These include using aqueous extracts of allelopathic plant species or mixing their residues in the soil to limit the growth of weeds and then improve the growth and productivity of the crop. For that some suggested the possibility of using aqueous extracts as natural, environmentally friendly pesticides as an alternative for chemical pesticides. They can be used directly in control or in developing new pesticides from them that are less harmful to the environment (Jamil, 2004).

This research aims to evaluate the allelopathic effect of aqueous extracts of Eucalyptus, Milk thistle weed and mallow on germination and early growth characteristics of Basil seeds also comparing it with their effect on Sow thistle and Arugula weed seeds in order to reach extracts that can be used in the biological control of weeds in the fields of medicinal plants.

2. Materials and Methods.

The experiment was conducted to study the allelopathic effect of some aqueous extracts of Eucalyptus, Milk thistle and mallow on the germination and growth of Basil, Arugula and Sow thistle seeds. The experiment was carried out in the laboratories of the Department of Field Crops – College of Agriculture at Tikrit University.

2.1. Preparation of the Aqueous Extract.

The aqueous extract was prepared at a concentration of 2% by taking 20 g of the plant residues of Eucalyptus, Milk thistle and mallow, and they were mixed with one liter of distilled water according to the method of (Mersie and Singh, 1987a). Then the mixture consisting of water and plant powder was put in an electric blender for a quarter of an hour after that each sample was filtered through three layers of gauze pieces. Then the solution was filtered using filter paper type Whatman NO. 1. After that the solution for each type of plant residues was placed in dark, tightly closed containers and kept in the refrigerator at a temperature of 5 °C until use (El-Khawas & Shehata, 2005) ^[6].

2.2. Planting Method.

This method was followed to study the allelopathic effect of some aqueous extracts of Eucalyptus, Milk thistle, and mallow on the germination and growth of Basil, Arugula and Sow thistle seeds using Petri dishes with a diameter of (13.8) cm. In each dish (10) seeds of the types used in the experiment (Basil, Sow thistle and Arugula seeds) were placed individually with three dishes for each treatment. Then

the seeds were placed on two filter papers and 10 ml of the prepared extract was added (in C at a temperature of 25 °C. After eight days of planting, the germination percentage was calculated and after 14 days the radicle was separated from the plumule for the growing seedlings as their lengths were measured then they were dried in an electric oven at 70 °C for 72 hours to measure their dry weights (Saleh, 2009). The treatments applied in the experiment, with a total of 12 dishes. The treatments with symbols were:

1. S1=Aqueous extract of Eucalyptus plant with Sow thistle weed.
2. S2=Aqueous extract of Milk thistle with Sow thistle weed.
3. S3=Aqueous extract of mallow with Sow thistle weed.
4. S4=Control treatment (Control) with Sow thistle weed.
5. S5=Aqueous extract of Eucalyptus plant with Arugula weed.
6. S6=Aqueous extract of Milk thistle with Arugula weed.
7. S7=Aqueous extract of mallow with Arugula weed.
8. S8=Control treatment (Control) with Arugula weed.
9. S9=Aqueous extract of Eucalyptus plant with Basil plant.
10. S10=Aqueous extract of Milk thistle with Basil plant.
11. S11=Aqueous extract of mallow with Basil plant.
12. S12=Control treatment (Control) with Basil plant.

2.3. Laboratory Studied Traits.

1. Seed Germination Percentage %: The percentage of germinated seeds in each treatment was calculated 8 days after planting. The germination percentage was calculated using the following formula:

$$\text{Germination Percentage} = (\text{Number of Germinated Seeds} / \text{Number of Seeds}) * 100$$

(Saied, 1984).

2. Plumule Length (cm): Measuring the plumule length from the beginning of the seed to its end (Saleh, 2009).
3. Radicle Length (cm): Measuring the radicle length from the beginning of its emergence from the seed to its end (Ali, 1982).
4. Dry Weight of Plumule (g): The dry weight of the plumule was calculated. It was dried in an electric oven at a temperature of 70 °C for 72 hours, then the weights were taken by a sensitive balance (ISTA, 2012).
5. Dry Weight of Radicle (g): The dry weight of the radicle was calculated. It was dried in an electric OVEN at a temperature of 70 for 72 hours, then the weights were taken by a sensitive balance (ISTA, 2012).

The results of the factorial experiment were analyzed according to a Completely Randomized Design (C.R.D) using the SAS statistical analysis program. The arithmetic means were compared using Duncan's Multiple Range Test (independent comparisons) at a probability level of 0.05.

3. Results and Discussion:

3.1. Germination Percentage.

Table (1) show that the presence of significant differences between treatments in this trait. The results appears that the plant extract of Eucalyptus had a negative effect on the seed germination of the studied weeds (Sow thistle and Arugula), and recorded the lowest mean which was 46.67 and 53.30%.

respectively. Also we notice that the control treatment for Sow thistle, Arugula and Basil seeds recorded a germination percentage of 100%. This points to possibility of these extracts act to inhibit weed growth. The results show that the plant residue extracts have shown an inhibitory activity on the growth of weeds.

The inhibition in germination percentage may be attributed to the fact that plant extracts contain allelopathic compounds such as phenolic acids, flavonoids, and terpenes. These compounds can work to inhibit water absorption and inhibit the activity of germination enzymes such as alpha -amylase and protease, which are responsible for the breakdown of stored materials in the seed. They also cause an increase in cell membrane permeability and leakage of ions, leading to germination failure. Eucalyptus extract was the most inhibitory may because it contains 1,8-cineole and alpha -pinene which have high comparative inhibitory activity (Rice, 1984; Einhellig, 1996) ^[14].

These results agree with El-Khawas and Shehata (2005) ^[6], who found that Eucalyptus extract reduced the germination percentage of *Vigna sinensis* and *Zea mays* by 40-45%. They also agree with Rice (1984) who mentioned that the intensity of inhibition is directly proportional to the concentration of allelopathic compounds. This may be attributed to the fact that Eucalyptus contains phenolic compounds and volatile oils such as 1,8-cineole, which work to inhibit cell division and destroy cell membranes (Singh *et al.*, 2020).

Table 1: Effect of spraying with aqueous extracts of some plant species on some weed seeds and Basil plant in traits of germination percentage, plumule length and radicle length.

Treatment	germination percentage %	plumule length (cm)	Radicle length (cm)
S1	46.67 f	3.167 c	1.22 f
S2	76.67 b	4.467 ab	2.453 d
S3	63.33 c	2.00 d	0.467 g
S4	100 a	4.00 ab	2.00 e
S5	53.30 e	2.177 d	1.267 f
S6	60.0 d	4.430 ab	3.867 b
S7	70.0 bc	2.833 cd	1.433 f
S8	100 a	4.500 ab	4.733 a
S9	76.67 b	3.997 ab	1.887 e
S10	66.67 cd	4.833 a	3.767 b
S11	63.33 cd	3.99 ab	3.167 c
S12	100 a	3.80 b	3.867 b

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

3.2. Plumule Length (cm).

Table (1) shows high significant differences between the aqueous extract treatments in the trait of plumule length for the studied plant species. The table clarifies that there is a significant inhibitory effect on the growth of plumule length for weeds studied in the experiment. The treatment of adding mallow extract to Sow thistle seeds adding Eucalyptus extract to Arugula seeds, as for mallow extract to Arugula seeds recorded the lowest rate for plumule length by reaching (2.00, 2.177, and 2.833 cm) respectively. Also we notice that when adding the aqueous extracts (Milk thistle, Eucalyptus, and mallow) to Basil seeds they recorded the highest rates which recorded 4.833, 3.997, and 3.990 cm respectively.

The reason for the inhibition of plumule length in these treatments can be attributed to the effect of phenolic compounds and alkaloids present in plant extracts, on the

process of cell division and elongation in the meristematic zone of the stem. These compounds reduce the chlorophyll content and so can inhibit the photosynthesis process which reflects negatively on the vegetative growth. However the increase in the plumule length of Basil when treated with Milk thistle extract is may due to the fact that the extract contains natural growth regulators like auxins and gibberellins in low concentrations which can work to stimulate cell elongation (Dayan *et al.*, 2009; Taiz, 2010) ^[3, 6]. These results agree with Dayan *et al.* (2009) ^[3], who mentioned that plant extracts contain compounds that inhibit the vegetative growth of plants.

3.3. Radicle Length (cm).

The results in Table (1) showed a reduction in the radicle length of the weed seedlings (Sow thistle and Arugula) under the effect of the aqueous extracts of the plant residues of Eucalyptus, Milk thistle and mallow. when comparing the means of the extract types we can find that the mallow extract and Eucalyptus extract inhibited the radicle growth of Sow thistle seeds and the means for each of them were 0.467 and 1.220 cm respectively. Through the table we notice that Arugula seeds were affected when adding Eucalyptus extract and mallow extract, as they recorded their lowest means which were 1.267 and 1.433 cm respectively. Also we notice a decrease in the radicle length of Basil when adding Eucalyptus extract which gave 1.887 cm. The reason for the inhibition of Sow thistle and Arugula radicle may be attributed to the fact that the radicle is the first part that emerges from the seed and touches the extract. There for it is considered the most sensitive to allelopathy. This inhibition occurs as a result of destroying the root cap cells, as the volatile terpene compounds like 1,8-cineole in Eucalyptus dissolve the lipids of the root cap cell membrane so they die, and therefore without the root cap radicle cannot penetrate the soil (Li *et al.*, 2019). These results agree with a study founded by Oueslati (2003) which said that lettuce radicle decreased to 90% with 10% Eucalyptus extract. They also agree with Einhellig (1996) ^[5] study who clarified that allelochemicals directly affect the root tip and cause inhibition in radicle length by a percentage ranging between 60-80% and agree with Qasem and Foy (2001) ^[8] as they mentioned that weeds secrete compounds that can cause inhibition to neighboring roots and cause symptoms of yellowing and stunting.

3.4. Dry Weight of Plumule (g).

It appears from Table (2) that the trait of plumule dry weight for Basil plant that there are significant differences at the 5% probability level between the aqueous extract treatments in the trait of plumule dry weight for Basil plant. We notice the significant superiority of the Milk thistle extract treatment by giving the highest trait mean which achieved 0.0233 g. while mallow and Eucalyptus extracts recorded 0.01667 and 0.01633 g respectively followed by the control treatment which recorded the lowest values of 0.0160 g. Maybe the reason for the significant stimulation in the plumule dry weight when treated with Milk thistle extract is due to its content of natural growth regulators such as auxins, gibberellins, and cytokinins. These regulators work to increase cell division and elongation in the meristematic apex and increase dry matter accumulation through improving the efficiency of the photosynthesis process and increasing chlorophyll content (Taiz, 2010) ^[6].

Perhaps the reason for the slight and non-significant inhibition in the plumule dry weight when treated with Eucalyptus and mallow extracts is due to their containing of allelopathic compounds such as 1,8-cineole, phenolic acids along with flavonoids. These compounds can lead to the inhibition of the photosynthesis process and reducing the efficiency of nutrient absorption which reflects negatively on the dry matter accumulation (Singh, 2003) ^[11]. These results agree with what was stated by both Taiz and Zeiger (2010) ^[12] on the role of auxins and gibberellins in increasing dry weight.

Table 2: Effect of spraying with aqueous extracts of some plant species for fenugreek plant on the traits of plumule and radicle dry weight for Basil plant.

Treatment	plumule dry weight (g)	Radicle dry weight (g)
S9	0.01633 b	0.005 a
S10	0.02333 a	0.00733 a
S11	0.01667 b	0.005 a
S12	0.0160 b	0.00633 a

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

3.5. Dry Weight of Radicle (g).

The results of Table (2) about the trait of radicle dry weight for Basil plant show no significant differences between the treatments. The absence of significant differences in the radicle dry weight may attributed to the fact that the root is considered the sensitivity of organ to allelopathic compounds compared to the plumule. Where the allelopathic compounds work to inhibit cell division in the root tip also reduce water and nutrient absorption and increase the permeability of root cell membranes. so the effect of the different extracts led to a closeness between them and the non-appearance of significant differences (Einhellig, 1996) ^[5].

3.6. Dry Weight of Sow thistle Weed (g).

Table (3) indicates the presence of a significant inhibitory effect on the dry weight of Sow thistle seedlings. The treatment of Milk thistle aqueous extract recorded the highest dry weight for Sow thistle plant reaching 0.02133 g and it significantly excelled over all treatments. while the treatment of Eucalyptus and mallow gave 0.01333 g for both of them, while the control treatment recorded the lowest dry weight by reaching 0.01133 g. The significant stimulation in the dry weight of Sow thistle plant when treated with Milk thistle extract is attributed to its containing of natural growth regulators such as auxins and gibberellins which can work to increase cell division and elongation and stimulate the photosynthesis process. Also the fact that Milk thistle contains nutrient elements and phenolic compounds in low concentrations may works to increase dry matter accumulation and improve the efficiency of absorbing elements from the soil (Taiz, 2010) ^[12]. The absence of significant differences between the Eucalyptus and mallow treatments and the control may be due to the fact that these two extracts contain allelopathic compounds such as 1,8-cineole, phenolic acids and alkaloids. Since these compounds work to inhibit the photosynthesis process and reduce the efficiency of water and nutrient absorption which leads to reducing the accumulation of dry matter (Singh *et al.*, 2003) ^[11].

These results agree with what was mentioned by Taiz and

Zeiger (2010) ^[12] on the role of growth regulators in increasing dry weight through stimulating cell division. They also agree with Singh *et al.* (2003) ^[11] who indicate that phenolic acids and terpenes reduce dry weight due to their negative effect on photosynthesis.

Table 3: Effect of spraying with aqueous extracts of some plant species on the trait of dry weight for Sow thistle weed.

Treatment	Dry weight (g)
S1	0.01333 b
S2	0.02133 a
S3	0.01333 b
S4	0.01133 b

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

3.7. Dry Weight of Arugula Weed (g).

The results of Table (4) clarified the presence of significant differences between the aqueous extract treatments in the trait of dry weight for Arugula seedlings. The Milk thistle extract treatment excelled by recording its highest trait mean (0.0390 g), while the Eucalyptus extract treatment recorded 0.01333 g and control treatment recorded its lowest mean which was 0.01033 g. The significant increase in the dry weight of Arugula seedlings when treated with Milk thistle extract may be due to its containing of natural growth regulators such as gibberellins, auxins, and cytokinins in optimal concentrations. These regulators can increase cell division and elongation and increase leaf surface area, therefore increasing the efficiency of the photosynthesis process and dry matter accumulation. Also the presence of micro and macro elements in the Milk thistle extract contributed to improving plant nutrition (Taiz, 2010) ^[6].

Maybe the reason for the severe significant inhibition in dry weight when treated with Eucalyptus extract is due to its containing of high percentages of allelopathic compounds such as 1,8-cineole, terpenes also phenolic acids. These compounds may lead to the inhibition of respiration enzymes reducing the permeability of cell membranes and inhibiting the photosynthesis process which reflects negatively on the dry matter accumulation (Singh *et al.*, 2003) ^[11].

The slight inhibition in dry weight when treated with mallow extract may be attributed to its containing of phenolic compounds and alkaloids in concentrations lower than Eucalyptus. These compounds can work on partial inhibition of metabolic processes without reaching the limit of severe inhibition (Duke *et al.*, 2000) ^[4]. Comparison with sources. These results also agree with Taiz and Zeiger (2010) ^[6] on the role of gibberellins and auxins in increasing dry weight and with Singh *et al.* (2003) ^[11] who mentioned that 1,8-cineole present in Eucalyptus causes strong inhibition of vegetative growth.

Table 4: Effect of spraying with aqueous extracts of some plant species on the trait of dry weight for Arugula weed.

Treatment	Dry weight (g)
S5	0.01333 d
S6	0.03900 a
S7	0.01933 c
S8	0.0220 b

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

Conclusions

Aqueous extracts from Eucalyptus, Milk thistle, and Mallow plants demonstrated distinct allelopathic effects on plant germination and early growth. Eucalyptus extract showed the highest inhibitory capacity, while Milk thistle acted as a growth stimulator for Basil, with the radicle identified as the most sensitive organ to these compounds.

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How to Cite This Article

Ismael OF, Ahmed RH, Hameed NA. The allelopathic effect of some aqueous extracts of Eucalyptus, Milk thistle, and Mallow on the germination and growth of Basil, Arugula, and Sow thistle seeds. *Int J Agric Nat Farm Res.* 2026;2(3):10-14. doi:10.54660/IJANFR.2026.2.3.10-14.

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