# Effect Concentration of Water Extract of Nerium oleander and Juglans nigra L. Allelopathy on Seed Germination and Radicle Length of (*Silybum marianum*)

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**Abstract**

An experiment was done to determine the allelopathic effects of Juglans nigra L. and Nerium oleander on the germination and radicle length of Milk thistle (*Silybum marianum*). The experiment was designed according to a completely randomized design (CRD). In this experiment, leaf-, hull-, and root extracts in two concentrations control, 5 and 10g L-1 was utilized. The experiment was conducted in a growth chamber with a temperature of 15 C0. After storing the seeds for 14 days, many characteristics were examined, including radicle length cm and germination rate percent. Concentrations of walnut and oleander extract had a substantial impact on every parameter under investigation. The results showed that the (T1) control treatment had the greatest germination and radicle length which were 70% and 20.08, respectively, compared with other treatments. A 10g L-1 extract concentration was used to measure the minimum values for two of the aforementioned parameters. It was found that (*Juglans nigra* L.) and Nerium oleander %10 extracts inhibit the growth of *Silybum marianum* and can be utilized to manage it in the field. However, more studies are needed to confirm the results.

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## Introduction

Allelopathy is a common biological phenomenon whereby the biochemical produced by one organism impacts the growth, development, and reproduction of other organisms. These biochemicals, also known as allelochemicals, can benefit or harm the target organisms (Cheng and Cheng, 2015). Most of these phytotoxic chemicals are known to be released by plants in an effort to stop the germination or growth of other plants. Plants produce 10,000 different chemicals, according to estimates, to defend themselves from disease, pests, and other plants, especially weeds (Kadioglu and Yanar, 2004). Currently, only 3% of phytotoxic chemicals are known to exist (Khanh *et al*., 2005). The roots stems, rhizomes, flowers, and leaves of plants all contain these allelochemicals (Amoo *et al*., 2008). As our understanding of these compounds expands, we may be able to use them as herbicides, which would be great for the environment (Kadioglu and Yanar, 2004). Walnut (*Juglans nigra* L.), which produces hydrojuglone, a non-toxic colorless chemical, is one of the most well-known allelopathic plants. According to Ercisli and Turkkal (2005) hydrojuglone can be found in leaves, stems, fruit hulls, inner bark, and roots. Hydrojuglone, an allelochemical, oxidizes and changes into the extremely toxic allelochemical juglone when it comes into contact with soil or air compounds (Appleton *et al.*, 2000). The nerium oleander is an evergreen shrub that can grow well in arid conditions and with a limited water supply. It may also flourish in unfavorable terrain. Because of this, it is available everywhere (Uslu *et al.*, 2018). It has long been believed that nerium oleander is poisonous due to the negative effects of the chemical compounds it produces. It has been shown to have an allelopathic effect on numerous weedspecies (Farooqui and Tyagi, 2018) Allelopathic glycoside and flavonoid compounds have been known to be released by this plant (Mojarad *et al.*, 2013). To lessen these losses, a variety of chemicals are being used. These chemical uses do, however, come with some undesirable side effects (Kadioglu and Yanar, 2004). The ecology is harmed and pest resistance is encouraged by the constant use of large amounts of pesticides. In agricultural systems, three million tons of herbicides are used annually (Bhadoria, 2011). To develop plant protection methods that are environmentally friendly, numerous studies have recently been carried out (Kadioglu and Yanar, 2004).

The annual milk thistle (*Silybum marianum*), which can reach heights of three meters and is a member of the largest plant family, the Asteraceae (Compositae), distinguishes itself from other weeds with its reddish-purple blooms, large leaves, and thorny stem (Shakeel and Yar, 2014). The majority of temperate regions of the world have this weed growing there. It is also categorized as a noxious weed. Kurdistan has a lot of milk thistle, according to (Sultana and Asaduzzaman, 2012). The objective of this study was to determine the allelopathic potential of *Nerium oleander* and *Juglans nigra* L. on seed germination and radicle length of milk thistle under laboratory conditions.

## Materials and methods

Laboratory experiments were conducted during 2021-2022 at the College of Agriculture, Raparin University.

## Collection of weed seeds

Seeds of weed Milk Thistle were collected from Ranya and Qalladiza district in the same season. A healthy and uniformly sized seed was chosen, sterilized with 10% sodium hypochloride for 10 minutes, and then washed repeatedly with distilled water to remove any remaining chemicals before sowing.

## Preparation of the aqueous extracts

The aqueous extract was prepared from fresh leaves and fruit hulls of the walnuts and leaves and roots of oleander plants. These materials were dried at room temperature and then grounded into powder. 5gm and 10gm of dried sample dissolved into 100 ml of distilled water and kept in water both of 40° for 2h. Then overnighted at 4°. The resulting greenish and dark extracts were filtered through filtered paper and stored in bottles (Javaid *et al.*, 2006). Thus 5% and 10% extract was obtained.

## Seed Germination and Seedling Growth

Ten surface sterilized seeds (*Silybum marianum*) were placed in a Petri dish 9 cm in diameter on double- layered filter paper. 8 mL of extract solutions were added to each Petri dish. While for control treatment distilled water was used. The Petri dishes were placed in a growth chamber at 15 C°. It was kept for 14 days. When the radicle first appears, seeds are deemed to have germinated. The number of seeds that germinated was counted, and the length of the radicle was measured with a millimeter ruler to determine germination. The percentage of germination was calculated as by (Chopra *et al.*, 2017).

**Germination**

$$\textbf{(\%)} = \frac{\textit{Number of germinated seeds}}{\textit{Total number of seeds tested}} \times 100$$

## Statistical analysis

The experimental design was arranged in a completely randomized design with three replicates. Data were analyzed statistically using analysis of variance with SPSS. Comparisons the treatments means were compared the mean using a Least Significant Differences Test (L.S.D).

**Table 1. Description of Treatments**

| Treatments | Description |
|---|---|
| $T_1$ | Control |
| $T_2$ | 5% *Nerium oleander* dry leaves extract |
| $T_3$ | 10% *Nerium oleander* dry leaves extract |
| $T_4$ | 5% *Nerium oleander* dry roots extract |
| $T_5$ | 10% *Nerium oleander* dry roots extract |
| $T_6$ | 5% *Juglans nigra* L. dry leaves extract |
| $T_7$ | 10% *Juglans nigra* L. dry leaves extract |
| $T_8$ | 5% *Juglans nigra* L. dry hulls extract |
| $T_9$ | 10% *Juglans nigra* L. dry hulls extract |

## Results and discussion

### Effect of walnut on Seed Germination

Data in Table 2, the effect of walnut extraction on Milk Thistle germination. Milk Thistle germination inhibition was increased when the extract concentration was increased. In this study, the control treatment $T_1$ had the highest germination rate of 70%. However, hulls extract 10% ($T_9$) showed the lowest germination rate 1.5. The high concentration of hull extract's inhibitory effect may be the cause of this decline in germination. Results were supported by the findings of (Mokhtari Karchegani *et al.*, 2014) who reported that germination percentage decreased as concentration increased. Sorghum extracts at concentrations of 15 and 20% reduced milk thistle seed germination to 69.70 and 60.60%, respectively, when compared to control treatment. (Efiong *et al.*, 2020) argued that the amount of phytochemicals in liquid and dry forms varies. Wet samples are more viscous and lack secondary metabolite separation according to the polarity of the solvent used.

**Table 2. Effect of walnut extractions on milk thistle seeds germination**

| **Treatments** | **Concentrations of extract** | **Germination rate** | **Germination percentage** |
|---|---|---|---|
| **Control** | 0 | 7 | 70% |
| **Leaves** | 5% | 4.5 | 45% |
| | 10% | 3.5 | 35% |
| **Hulls** | 5% | 2.5 | 25% |
| | 10% | 1.5 | 15% |
| **$LSD_{(0.05)}$** | 0.95 | | |

### Effect of Nerium on Seed germination

*Nerium oleander* has an allelopathic effect on milk thistle seed germination, as indicated in Table 3. The aqueous Nerium Leaf extract and *Nerium oleander* root extract clearly prevented Milk Thistle seeds from germinating. The maximum seed percentage was shown in the control where no extract was used.

Which was 70%. The highest inhibitory effect was found in the root extract 10% ($T_5$) treatment which was 7.5%. (Al-Samarai *et al.*, 2018) who discovered that the highest amount of germination inhibition was seen in soil irrigated with a crude extract from Nerium. Eighty percent of plants were inhibited. Uslu *et al.* (2018) reported that the control treatment had the highest germination rate (93.33%). However, a decrease in germination percentage was observed as the concentration of Nerium extract increased (Begam *et al.*, 2020) Thevetia peruviana and *Nerium oleander* vinegar leaf extracts in various concentrations and formats had a significant impact on the germination and seedling growth of purple nutsedge

**Table 3. Effect of nerium extracts on milk thistle seeds germination**

| Treatments | Concentrations of extract | Germination rate | Germination percentage |
|---|---|---|---|
| **Control** | 0 | 7 | 70% |
| **Leaves** | 5% | 5.5 | 55% |
| | 10% | 2 | 20% |
| **Roots** | 5% | 2.5 | 25% |
| | 10% | 0.75 | 7.5% |
| **$LSD_{(0.05)}$** | 1.49 | | |

## Effect of walnut on length of radicle (cm)

The concentration of walnut extract also significantly reduced the radicle length of (*Silybum marianum*) (Table 4). The maximum radicle length in the control treatment was 20.08, and it got shorter as the concentration went up. This may be due to the fact that releases allelochemical substances into the environment, such as tannins, wax, flavonoids, glycyrrhizic acid, and phenolic acids, which significantly inhibit the growth and development of nearby weeds and plants. (Appleton *et al.*, 2000) Juglone is not very mobile in the soil and has low water solubility, but even small amounts can harm delicate plants. When a plant root is within 0.5 to 0.25 enhance of a walnut root, it can come into contact with juglone. (Mustafa *et al.*, 2017) It was made clear that the root length was significantly reduced by the highest concentration of 50% aqueous liquorice extract when compared to the treatment using distilled water, which recorded the longest root length of 8.431 cm.

**Table 4. Allelopathic effect of walnut water extracts on radicle length of milk thistle**

| Treatments | Concentrations of extract | Radicle Length |
|---|---|---|
| **Control** | 0 | 20.08 |
| **Leaves** | 5% | 9.18 |
| | 10% | 4.3 |
| **Hulls** | 5% | 3.98 |
| | 10% | 1.3 |
| **$LSD_{(0.05)}$** | 2.47 | |

## Effect of Nerium on length of radicle (cm)

The study of Table 5 revealed that the Nerium oleander decreased the Milk Thistle radicle length as compared to the control. The smallest root length was registered in the root extract (10%) ($T_5$) treated seeds which was 2.8, which significantly differed from the other extract. The highest root length was obtained in the control. This decrease in radicle length might be due to an inhibitory effect of the extract of Nerium on the growth of this weed. Maximum radicle length was recorded in control treatments which decreased with an increase in concentration (Uslu *et al.*, 2018). The findings and the results were in agreement (Jabran and Farooq, 2013; Jurewicz *et al.*, 2012), showed that the water extract of several plant extracts had a favorable effect on the prevention of seed germination, seedling length, and dry stem weight of all the weeds investigated.

**Table 5. Allelopathic effect of nerium water extracts on radicle length of milk thistle**

| Treatments | Concentrations of extract | Radicle Length |
|---|---|---|
| **Control** | 0 | 20.075 |
| **Leaves** | 5% | 11.9 |
| | 10% | 9.15 |
| **Roots** | 5% | 5.625 |
| | 10% | 2.8 |
| **LSD $_{(0.05)}$** | 2.73 | |

## Conclusion

Overall application of Walnut and Nerium extracts at various concentrations suppress germination and radical length related parameter of Milk thistle. So it can reduce the milk thistle population in a given environment.

## Conflict of interest

The authors declare there are no conflicts of interest among all authors.

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