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Effects of Different Concentrations of Leaf Aqueous Extract of *Tribulus terrestris* L. on the Germination of Economic Seeds

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Abstract

Tribulus terrestris herb possesses various biological activities due to the active substances in its composition. This study aimed to evaluate the potential effect of the aqueous extract of the leaves of *Tribulus terrestris* L. on T*riticum aestivum, Hordeum vulgare, and Vici faba* seeds under laboratory conditions. The effect of various concentrations (5%, 3%, 1%, and 0.5%) on germination and seed growth was observed and compared to the control (distilled water). The concentrations of the aqueous extract had an allelopathic effect on imbibition, germination rate, radicle length, and plumule length. It was noticed that the rate of inhibition increased with the increasing concentration. Overall, the active substances in the aqueous extract of the leaf of the *Tribulus terrestris* L. plant had an allelopathic effect that increased with increasing concentration.

Article Info

Keywords:
Tribulus terrestris L., Leaves Aqueous Extract, Allelopathy, Economical Seeds, Growth parameters

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1. INTRODUCTION

Tribulus terrestris is a plant found worldwide, particularly in South Africa, Asia, and India. This plant typically grows in arid and sandy soil. It belongs to the Zygophyllaceae family, which comprises of 25 genera and 250 species.

It is a crawling herb with stems that can grow up to 70 cm in length, with opposite and unequal leaves and small yellow flowers. Traditional Indian, Korean, and Chinese medicines have various applications for this plant. Humans contain several active substances including alkaloids, saponins, tannins, and polyphenols. The active substances in this plant vary based on their geographical location and season [1][2][3].

Additionally, a study by [4] indicated that the composition of saponins differs according to the environmental conditions under which the plant grows. Conflict of Interest declaration: The authors declare that they have no affiliations with or involvement in any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

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These active substances have many biological activities, such as the inhibition of digestive enzymes [5] and antibacterial effects against Gram-positive and Gram-negative bacteria [6].

It is also used to treat renal dysfunction [7]. Plant extracts offer a safe and eco-friendly substitute for ersatz chemical pesticides, benefiting the environment and human health [8].

Certain plant extracts have a fertilizing effect that stimulates germination and growth without causing harm to the environment or human health [9].

However, some plant extracts have allelopathic effects, according to [10].

An allelopathic effect is the effect of one plant on another, which can be beneficial or harmful. *Tritium aestivum* and *Hordeum vulgare* seeds are among the oldest crops consumed, both globally and locally.

Both belong to the Poaceae family. One of the characteristics of this family is that it tolerates different environmental conditions and increases the organic content

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of soil [11] and [12].

Vicia faba seeds are also considered one of the world's oldest and most widely consumed crops, belonging to the Fabaceae family [13].

They are also important plants that can fix nitrogen in the soil. Therefore, they are often grown in rotation with cash crops [14].

This study aimed to evaluate the effects of various concentrations of *Tribulus terrestris* L. aqueous extract on the germination and growth of *Tritium aestivum, Hordeum vulgare, and Vicia Faba* seeds.

2. MATERIALS AND METHODS

2.1. Preparation of Leaves Aqueous Extract from Tribulus terrestris L. :

2.1.1. Drying Process

Fresh leaves of *Tribulus terrestris* plants were collected from the Al-Sabean area of Sana'a city, which is an arable area. The taxonomy of the *Tribulus terrestris* was carried out with the assistance of Dr. Hassan Ibrahim, Professor of Plant Taxonomy, Botany Section-Department of Biology, Faculty of Science, Sana'a University, Yemen.

A plant sample is stored in the herbarium of the Faculty of Science at Sana'a University, identified by herbarium number 1176. All practical work was performed at the Biological Sciences Laboratories of the Faculty of Science at Sana'a University.

Leaves were washed and dried at room temperature to avoid any effects on the composition of the active substance. The drying process was continued for 10 days, after which it was ground and placed in airtight containers.

2.1.2. Extraction Process

The extraction was performed as described in [15], with some modifications. Fifty grams of leaf powder were dissolved in 500 ml of distilled water.

An Erlenmeyer flask was placed on a shaker at 120 rpm for 24 h, filtered through Whatman No. 1 filter paper, and centrifuged for 15 min, followed by another filtration process using Whatman No.

1 filter papers. After filtration, the extract was placed in glass dishes in an oven at 40 ℃ for 24 h to dry. The extracts were packaged in dark glass bottles and stored at 4℃. From this stock, 0.5, 1, 3, and 5% were prepared for further use.

2.2. Colorimetric detection of active substances.

2.2.1. Flavonoids

In a test tube, 2 mL of *Tribulus terrestris* leaf extract in drops of NaOH was yellow. Yellow indicates the presence of flavones [16].

2.2.2. Tannins

In a test tube, 5 mL of extract was placed, 1 mL of 5% ferric chloride solution was added, and the appearance of a blue-black color indicated the presence of tannins. [16].

2.2.3. Saponins

In a test tube, 5 ml of the extract was mixed with 5 ml of distilled water and shaken. The appearance of the foam and its persistence for 15 min were evidence of the presence of saponins. [16].

2.2.4. Alkaloids

We prepared a test tube with 1 ml of the aqueous extract and then added five drops of Wagner's reagent. Brown precipitate [16].

2.2.5. Phenols

This was accomplished by adding 1 % %drops of ferric chloride to a mixture of 50 mg extract powder and 5 ml of distilled water. [17].

2.3. Seeds preparation

Seeds were obtained from local stores. Seeds of similar size were selected, sterilized with sodium hypochlorite 0.5%, washed with distilled water [18], open-dried, and used. Seeds were soaked in distilled water for two hours [19] in nine cm-deep Petri dishes lined with filter paper (Whatman No.1), and the seeds were evenly distributed. Three replicates were made for each concentration (5%, 3%, 1%, and 0.5%), with distilled water as the control.

2.4. Seed Germination and Seedling Growth

The seeds were kept under suitable conditions of humidity and temperature (22 and 27 ℃). Imbibition was performed for 48 h. The germination rate was monitored, and radical and plumule measurements were recorded after 7 days.

2.5. Germination percentage

2.5.1. Imbibition:

The dry seeds were weighed using a sensitive balance and then re-weighed after soaking to calculate soaking imbibition. The following formula was used: **[Seed im-**

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bibition (%) =(W2 -W1)/W1] × 100] Where W1 is the weight of the seed before imbibition. W2 is the weight of the seeds after imbibition [20].

The imbibition process was observed for 48 h, and the imbibition time ended at root emergence [21].

2.5.2. Germination Rate

To calculate the final germination rate, the following formula by [22] was applied: **[GR: number of seeds germinated in 4 days/total number of seeds × 100].**

2.5.3. Radical & Plumule lengths

After 7 days, the radical and plumule lengths were measured using a millimeter ruler and the mean was calculated.

Statistical Analysis To determine the significant differences and statistical significance of the results, a statistical analysis was performed by two-way ANOVA using SPSS version 26.

3. RESULTS

3.1. Presence of main consistent color detection.

The aqueous leaf extract of *Tribulus terrestris* contained Saponins, Alkaloids, Flavonoids Phenols, and Tannins (Table 1) (Figure 1).

Table 1. Colorimetric detection of leaf aqueous extract of *Tribulus terrestris.*

Chemical constituent	Composition
Saponin	+
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+

Present +, Absent -

3.2. Imbibition and Germination Rate

The imbibition rate was inversely proportional to concentration; as the concentration increased, the imbibition rate decreased.

The effect occurred in the imbibition stage of *Tritium aestivum, Hordeum vulgare,* and *Vicia faba* seeds, where the highest percentage of imbibition rate was in favor of the control (85%,87%, and 93%, respectively).

The lowest percentage of imbibition was observed at 5% concentration (31%,35%, and 29%). At a concentration of 3%, the percentage of imbibition was (44%,42%,

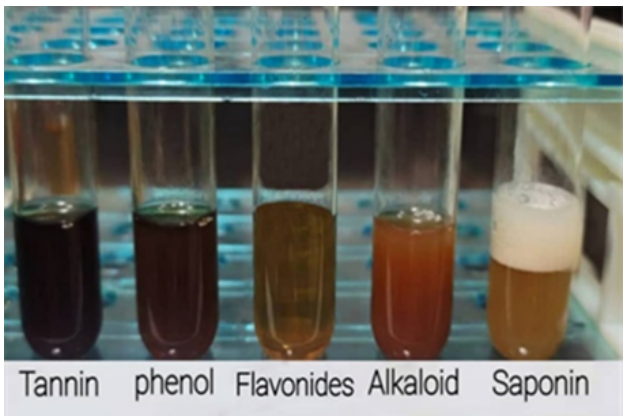


Figure 1. Colorimetric detection of leaf aqueous extract of *Tribulus terrestris*.

and 34%), with a concentration of 1% (49%, 48%, and 42%), while the concentrations of 0.5% the percentage was (56%,58%, and 51%) for *Tritium aestivum, Hordeum vulgare, and Vicia faba* seeds, respectively (Figure 2).

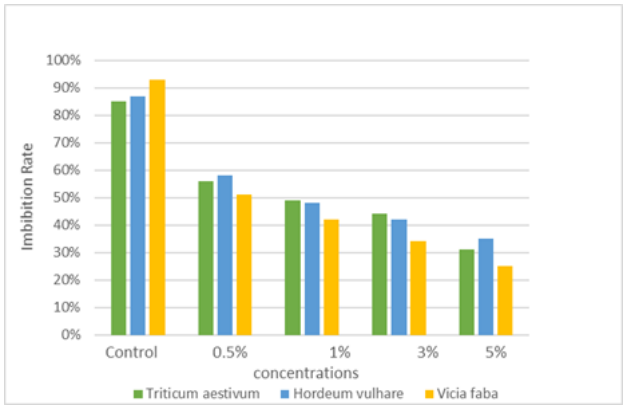


Figure 2. Imbibition rate using different concentrations of aqueous *Tribulus terrestris* leaf extract.

The germination rates of T. aestivum, H. vulgare, and V. faba were 0.5% (34%, 31%, and 37%) for *Tritium aestivum, Hordeum vulgare, and Vicia faba*, respectively). Concentration: 1% (28%, 22%, and 24%) for *Tritium aestivum, Hordeum vulgare, and Vicia faba,* respectively. Concentration: 3% (14%, 11%, and 12%) for *Tritium aestivum, Hordeum vulgare, and Vicia faba*, respectively. At a concentration of 5%, the germination rate was 0% for all the seeds.

These results were compared to those of the control (distilled water) (61%, 57%, and 65%) for *Tritium aestivum, Hordeum vulgare, and Vicia faba*, respectively. (Figures 3 and 4), respectively.

3.3. Length of radical and plumule

The different treatment concentrations influenced the average lengths of the radicle and plumule (Tables 2 and 3).

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Table 2. Effect of aqueous extract of *Tribulus terrestris* leaf on radicle length of *Triticum aestivum, Hordeum vulgare*, and *Vicia faba* seeds after 7 days.

Conc. / Seeds	Control	5%	3%	1%	0.5%
Tritium aestivum	2.9±0.05	0	0.5±0.07	0.9±0.05	1.3±0.05
Hordeum vulgare	3.5±0.04	0	0.6±0.05	0.9±0.04	1.7±0.06
Vici faba	2.3±0.05	0	0.5±0.06	0.7±0.05	1.5±0.04

Table 3. Effect of aqueous extract of *Tribulus terrestris* leaf on plumule length of *Triticum aestivum, Hordeum vulgare*, and *Vicia faba* seeds after 7 days.

Concs / Seeds	CONTROL	5%	3%	1%	0.5%
Triticum aestivum	7.1±0.05	0	0.7±0.04	1.9±0.04	3.9±0.04
Hordeum vulgare	10.3±0.03	0	1.8±0.04	2.7±0.06	4.2±0.07
Vicia faba	7.4±0.05	0	2.1±0.04	2.5±0.04	3.1±0.04

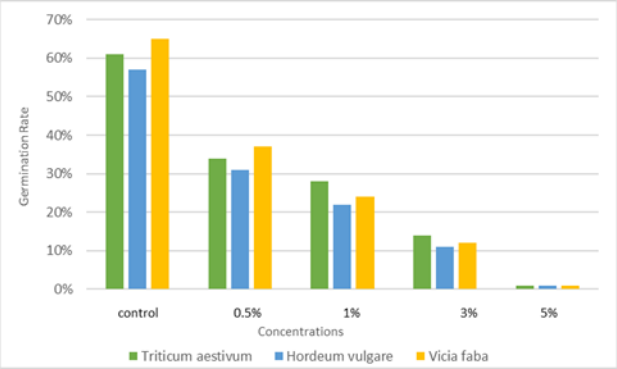


Figure 3. Germination rates using different concentrations of leaf aqueous extract of *Tribulus terrestris*.

For the control group, the average radicle lengths were 2.9 cm, 3.5 cm, and 2.3 cm for *Triticum aestivum, Hordeum vulgare, and Vicia faba*, respectively. Similarly, the average plumule lengths of these plants were 7.1 cm, 10.3 cm, and 7.4 cm,respectively. At a concentration of 3%, the average radicle lengths were 0.5 cm, 0.6 cm, and 0.5 cm, and the average plumule lengths for *Triticum aestivum, Hordeum vulgare, and Vicia faba* were 0.7 cm, 1.8 cm, and 2.1 cm, respectively. At a concentration of 1%, the radicle lengths were 0.9 cm, 0.9 cm, and 0.7 cm, and the corresponding average plumule lengths were 1.9 cm, 2.7 cm, and 2.5 respectively, for *Triticum aestivum, Hordeum vulgare, and Vicia faba*. As of the lower concentration of 0.5%, the average radicle lengths were 1.3 cm, 1.7 cm, and 1.5 cm, whereas the average plumule lengths were 3.9 cm, 4.2 cm, and 3.1 cm, for *Tritium aestivum, Hordeum vulgare, and Vicia faba,* respectively.

4. DISCUSSION

These results indicate that higher concentrations of the substances in the aqueous extract of *Tribulus terrestris* leaves reduce the imbibition process, eventually leading to a decreased germination rate as the concentration increases. This finding aligns with the research conducted in [23].

He stated that the underlying reason may be related to the retarded diffusion potential, which decreases the ability of seeds to absorb water due to reduced osmotic absorption strength within the seeds.

The active substances hinder the movement of water molecules and their transfer to the seeds, resulting in a lower rate of imbibition at higher concentrations but a higher rate of imbibition with the control (distilled water) and low concentrations.

This is consistent with the findings of [24] and [18], who indicated that exposure to various conditions diminished water uptake during imbibition. Another reason for the inhibitory effect on the imbibition process and germination rate may be due to the active substances present in the extract, such as tannins and saponins.

These active substances hinder the imbibition and germination process by inhibiting the action of the alpha-amylase enzyme, which is considered an allelopathic effect, especially saponins, which have been shown to have an inhibitory effect on enzymes.

This agrees with [5], who found that saponins can inhibit the action of the enzymes responsible for the digestion of starch and fats. According to colorimetric examination, the aqueous extract contains saponins and tannins, which are active substances that have allelopathic effects, as indicated by [25], who found that high

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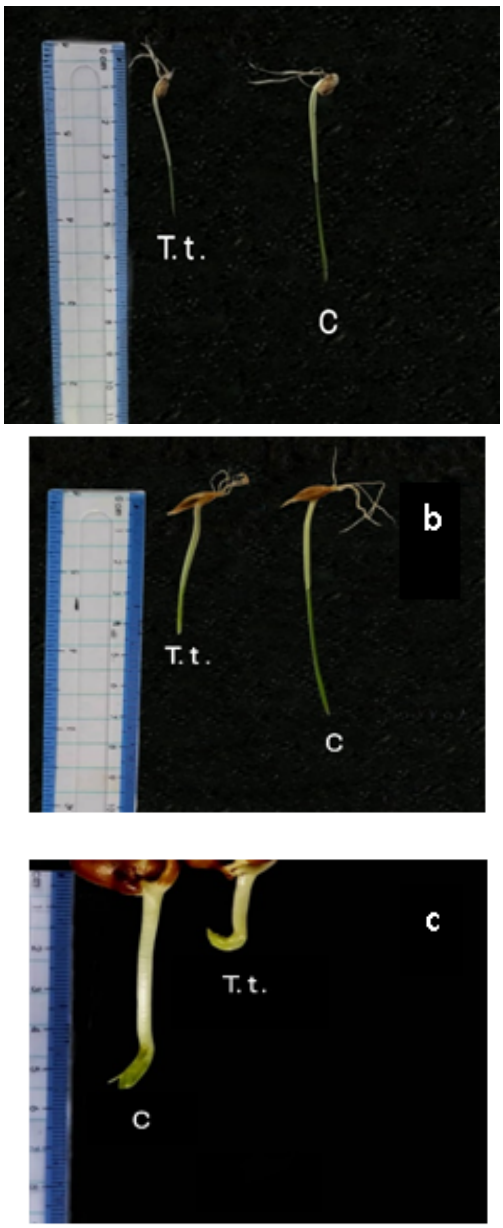


Figure 4. Length of the plumule with a concentration of 0.5% for : *a-Triticum aestivum, b-Hordeum vulgare, c-Vicia faba, Tribulus terrestris T.t., control C.*

concentrations of extracts of *Thunbergia laurifolia* Lindl.

leaves contain active substances such as saponins, tannins, and flavonoids that inhibit the alpha-amylase enzyme.

It was found that the high concentrations had an inhibitory effect on the germination and growth of some types of weed plants. It was also explained that the reason may be that the extracts contain active substances, such as saponins and tannins.

A study by [26] noted the same conclusion with the same plant)*Thunbergia laurifolia(*, which was found to contain saponins, tannins, alkaloids, and phenols that also contributed to an inhibitory effect on alpha-amylase activity.

All concentrations of *Tribulus terrestris* leaf extract inhibited germination and growth of *Tritium aestivum, Hordeum vulgare,* and *Vicia faba* seeds. The results of this study align with [27], in which the aqueous extract of *Tribulus terrestris* had an inhibitory effect in its different concentrations on the germination and growth of Maize and Wheat seeds. *Tribulus terrestris* contains active substances that have an allelopathic effect and inhibit seed germination.

Saponins are heterosides with surface-active properties. A foam solution was formed in water. These molecules significantly control interactions between plants and other competing plants [28].

According to a study by [29], saponins can interact with lipophilic and water-soluble compounds and penetrate biological membranes. The effect of saponins extends beyond cells and directly affects enzymes [30] and [5].

In another study,[31] it was found that tannins and saponins suppressed the activity of alpha-amylase. Additionally, [32] discovered that cinnamic acid amides, which are active substances found in *Tribulus terrestris* plants, can inhibit glucosidase. Glucosidases facilitate the breakdown of glycosidic bonds in oligosaccharides.

The results of this study align with earlier findings on *Tribulus terrestris* [33], which indicated that areas where the Tribulus terrestris plant is present have a lower percentage of annual plants. The presence of this plant also inhibited the germination and elongation of the roots of the surrounding annual plants compared with other species.

Similarly, [34] reported that an aqueous extract of the *Tribulus* plant inhibited germination. This finding is also supported by [27], who concluded that increasing the concentration of the extract had an inhibitory effect on both root length and plumule growth.

Generally, the results of this study showed the same consequence as in earlier studies, even at lower concentrations of the *Tribulus terrestris* aqueous leaf extracts

5. CONCLUSION:

This study evaluated the effect of an aqueous extract of *Tribulus terrestris* leaves on the seeds of *Triticum aestivum, Hordeum vulgare, and Vicia faba*.

The results showed that the seed imbibition rate, germination rate, and length of both the radical and plumule were negatively affected by all concentrations of the extract, with the most significant effects observed at the highest concentrations. Based on these findings, the extract may be beneficial as a natural pesticide for controlling harmful weeds.

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