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# Allelopathic potential of invasive alien plant species, *Xanthium strumarium* L., water *and* methanol extracts on germination and seedling growth of *Guizotia abyssinica* (L.f.) Cass., and *Linum usitatissimum* L.

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## Abstract

The objective of this study was to investigate the allelopathic impact of *Xanthium strumarium* on the seedling growth and germination of *Guizotia abyssinica* and *Linum usitatissimum*. Plant specimens and samples of *Xanthium strumarium* were collected from the Minjar-Shenkora district in Ethiopia. In the laboratory, a series of dilutions of extracts from the leaves and roots of *Xanthium strumarium* were prepared using distilled water and methanol (99.85%) as solvents. Twenty-five grams of root and leaf samples were soaked separately in 250 mL of the specified solvent to create the stock solution. The experiment was carried out using Petri dishes in a completely random manner, with three replications repeated twice. Various concentrations of the extracts (25, 50, 75, and 100%) were applied as treatments, while distilled water served as the control. Each petri dish contained 25 seeds of each test crop and 5 mL of the corresponding extract. The number of *Linum usitatissimum* and *Guizotia abyssinica* seeds germinating was recorded every 24 hours until 12 and 14 days, respectively. Both descriptive and inferential analyses were conducted. When *Guizotia abyssinica* and *Linum usitatissimum* seeds were treated with 100% concentrations of water extract from leaf samples, germination percentages were inhibited by 72 and 77.4%, respectively. In methanol extracts, the entire inhibition of germination was observed in both test plant species at ≥ 50% and ≥ 75% concentrations of leaf and root samples, respectively. Therefore, the inhibitory effect of water and methanol extracts showed a concentration-dependent pattern, with increasing concentrations resulting in greater inhibition. The inhibitory effect of leaf extracts was more pronounced compared to root extracts, suggesting the presence of herbicidal activities. Further phytochemical and molecular studies are

recommended to identify the active compounds in *Xanthium strumarium*. Additionally, utilizing isolated allelopathic substances as a means of controlling invasive alien species is suggested.

**Key words:** Concentration of extracts; distilled water; methanol; leaf and root samples

# 1. Introduction

Invasive Alien Species (IASs) are exotic species whose introduction and spread threaten biological diversity. IASs have been identified as the second cause of species extinction next to habitat deterioration, affecting the economy and environment. The effects of IASs on the environment are linked to alterations in ecosystem structure and functioning. The social aspect is usually associated with effects on human health and safety as well as on quality of life in general (Senator and Rozenberg, 2017). Invasive Alien Plant Species (IAPSs) are becoming the biggest challenge in farming systems globally, mainly in smallholder agricultural systems since all IAPSs are weeds, although not all weeds are IAPSs (Witt and Luke, 2017). The negative impact of IAPS on food security as well as economic performance is acutely felt in developing countries, where agriculture accounts for a higher proportion of gross domestic product (GDP) (Rai and Singh, 2020).

The susceptibility of cultivated plants to IAPSs can vary depending on the density and species of the IAPSs. *X. stamarium* is one of the most common IAPS in many crops. This IAPS affects plant growth, seed production, and biomass (Karimmojeni *et al.*, 2021). It is one of the worst IAPS in the world, with the common name cocklebur. *X. Strumarium* is an annual herbaceous plant belonging to the Asteraceae family. It is a universally known invader that has had significant negative biodiversity, economic, ecological, and social impacts in many areas of the world. It spreads quickly and outcompetes other native plant species (Ullah *et al.*, 2022). It invades farmland, roadsides, wetlands, disturbed land, fallow land, crops, plantations, drainage ditches, water courses, lowlands, floodplains, and overgrazed pasturelands. *X. strumarium* is one of the most widespread and abundant IAPSs in East Africa and Ethiopia (Witt and Luke, 2017). It is a major weed of crops such as soybeans, cotton, maize, and groundnuts that causes yield losses. Weeds with allelopathic potential have been affirmed to cause substantial destruction in agriculture, predominantly in smallholder farming systems (Hussain *et al.*, 2013; *et al.*, 2014; Laizer *et al.*, 2021).

Allelopathy is a biological marvel of chemical interaction among plants, and this marvel has great potential to be used as an effective and environmentally friendly tool for weed management in field crops. In field crops, allelopathy can be applied through intercropping, crop rotation, cover crops, mulching, and allelopathic water extracts to manage weeds. Accumulating evidence indicates that some plant species possess potent allelochemicals that have great potential to be eco-friendly natural herbicides (Khamare *et al., 2022).* Therefore, recent research considerations have concentrated on searching for other strategies for chemical weed control in several crops. Reduction in herbicide consumption is one of the key goals of current agriculture, and there is much importance placed on the search for substitute weed management strategies that are cheap, safe, and sustainable. Allelopathy is considered an effective, economical, and environmentally friendly weed management approach. The release of allelochemicals from leaves, flowers, seeds, stems, and roots of living and decomposing plant materials can influence weed density and growth (Bai *et al., 2022*).

*Xanthium strumarium* is a successful invader of different crops. One of the basic mechanisms that account for the IAPS' success is allelopathy (Kadioglu, 2004; Novak *et al.*, 2018). Thus, allelochemicals suppress germination, seedling growth, and reproduction of indigenous plant species in the invaded plant community (Maharjan *et al.*, 2007; Thiébaut *et al.*, 2019). Flowers, leaves, stems, roots, soil leachates, and their derived compounds can have allelopathic activity. Leaves may be the most consistent source of allelochemicals, followed by roots. Allelochemicals are discharged into the environment via exudation, volatilization, decomposition, and leaching (Singh *et al.*, 2021; Xie *et al.*, 2021). An understanding of the allelopathic potential of successful invaders contributes to numerous aspects of IAPS management and the development of IAPS policy and regulation (Inderjit, 1996; Trezzia *et al.*, 2016).

*Xanthium strumarium* is one of the most damaging IAPS in Ethiopia, posing significant threats to the country's biodiversity and habitats. This has resulted in the loss of numerous indigenous plant species that hold importance in Ethiopia's natural heritage. Despite its destructive impact, no previous studies have explored the allelopathic potential of *X. strumarium* on *G. abyssinica* and *L. usitatissimum.* Therefore, the aim of this study was to investigate the allelopathic effects of *X. strumarium* on the germination and seedling growth of *G. abyssinica* and *L. usitatissimum.* Studying the allopathic potential of IAPSs and determining their properties is very important because allelochemicals are believed to be better candidates for green, natural herbicides and weedicides that are proven to be environment-friendly, unlike synthetic chemicals (Chen *et al.*, 2017; Scavo *et al.*, 2019).

# 2. Materials and methods

To examine the effect of *X. strumarium* leaf and root aqueous extracts on the germination and seedling growth of *G. abyssinica* and *L. usitatissimum,* laboratory experiments were conducted at the Ethiopian Biodiversity Institute from December 2021 to June 2022. The samples, leaf and root, were selected for this investigation because they are known to be the sources of most of the allelochemicals in the plant (Zhang and Fu, 2010; Weston *et al.*, 2012). The specified samples were extracted using methanol (99.85%) and distilled water.

## Collection of plant materials

*Xanthium strumarium* leaf and root samples were collected from the Minjar-Shenkora district, Ameti Kebele (the smallest administrative zone in Ethiopia), approximately 135 km east of Addis Ababa (39°28'02.742" E, 8°56'24.942" N at 1760 meters above sea level) from December 11–13, 2021. A sharp pruning shear and hatchet were used to take the samples. Sterilization was conducted on the cutting edge of those tools using alcohol. The collected samples were placed in a moisture-free environment in loosely sealed plastic bags and quickly transported to the laboratory. The soil particles and other foreign materials were removed from the samples by gentle brushing. The samples were hacked into small pieces and air-dried in the shade at a room temperature of 25°C for about a month. The dried samples were ground in the laboratory using a mechanical grinder. Then, the powders were stowed in plastic carafes at 25°C until extraction (Dar *et al.*, 2017; Hassan *et al.*, 2018).

## Extract Preparation

Aqueous extracts of *X. strumarium* leaf and root samples were made in the laboratory. In conical flasks, 25 grams of each sample type were separately soaked in 250 mL of distilled water. The flasks were shaken at 150 rpm churning speed on an orbital shaker for 24 hours

at room temperature to achieve thorough extraction. To acquire an ultimate volume of 250 ml, each crude extract was filtered using Whatman filter paper no.1. Both crude extracts were diluted with distilled water in the ratio of: 25 mL extract: 75 mL distilled water, 50 mL extract: 50 mL distilled water, 75 mL extract: 25 mL distilled water, and 100 mL extract: 0 mL distilled water to obtain different concentrations of 25%, 50%, 75%, and 100%, respectively (Dar *et al.*, 2017; Singh *et al.*, 2021).

The samples were also extracted with methanol. Similar procedures were conducted for methanol extraction except for the elimination of the alcohol by evaporation. The methanol extracts were evaporated using a rotary evaporator at 40°C using Rota Vapor Buchi R-114, and the residues were weighed. A thick mass of coagulated liquid was then collected. From a 25-gram sample of powder, the average extract was 5.12 g after the completion of evaporation processes. For the bioassay, each stock extract from *X. strumarium* leaf and root samples was diluted in distilled water to generate extract serial dilutions of 25%, 50%, 75%, and 100%. All the aqueous extracts were stored in the refrigerator at 4° C until use (Mehrafarin *et al.*, 2011; Laizer *et al.*, 2021).

## Test Seed Preparation

In accordance with the Ethiopian genetic resources access and benefit sharing law, the test seeds were accessed from the Ethiopian biodiversity institutes which were collected from the Amhara area of the North Shewa Zone. The rationale behind choosing these native plant species was their ability to readily germinate, in addition to their great economic importance to the local community within the research location.

Different accessions (accession no. 10287, 13505, 13507, 18792, 18800, 18805, 18809, 18812, 18814, and 18816 for *L. usitatissimum* and 15117, 15124, 15138, 15139, 15204, 15565, 212198, 212494, 212500, and 218485 for *G. abyssinica)* of test plant species seeds were surface disinfected for 30 minutes in 5% sodium hypochlorite, then rinsed six times with distilled water (Turk and Tawaha, 2003; Elisante *et al.*, 2013). In this study, different accessions were used, which helped to identify accessions with high alleopathic potential. This is because different accessions might have different responses to alleleochemicals.

## Germination bioassay

Twenty-five (25) seeds of *L. usitatissimum* and *G. abyssinica* were placed in 9-cm-diameter Petri dishes lined with Whatman No. 1 filter paper. The Petri dishes were moisturized with 5 ml of different concentrations of water extracts from leaf and root samples of *X. strumarium* (25%, 50%, 75%, and 100%). When necessary, the Petri dishes were also moistened with distilled water. In all experiments, the control was only distilled water. Each treatment was repeated three times, and each of the three replicates was repeated twice. For the methanol extracts, the germination tests were also performed in Petri dishes on filter paper. Methanol extracts (5 ml) were applied to each individual filter paper. When the radicle grew to 2 mm long, the seeds were considered to have germinated. The number of seeds germinated was recorded every 24 hours until 12 and 14 days for *L. usitatissimum* and *G. abyssinica*, respectively (Stefanello *et al.*,2017; Hussain *et al.*,2020).

### Seedling length (cm)

The plumule and radicle lengths of test plant seedlings were measured using a ruler calibrated in cm, and the average length was taken from five seedlings from each treatment, which were selected randomly (Hussain *et al.*, 2020).

### Dry weight of seedlings (mg)

The dry weights of the test plant seedlings were measured using an electronic balance. The average dry biomass was then estimated in mg (5 seedlings from each treatment) (Benyas *et al.*,2010). The dry weight of the seedlings was measured after drying at 70°C for 48 h in an oven (ISTA, 2005).

### Experimental design and data analysis

The bioassay experiments were designed in a Completely Random Design (CRD), with three replications repeated twice to avoid any experimental error. The data was analyzed using the R package (version 4.2). The sprouting percentage and growth rate were examined by means of descriptive statistics. Using basic linear regression analysis, the significance of the effects of *X. strumarium* root and leaf extract on the germination and growth of the experimental crops was measured. The quantity of seeds germinated and seedling growth per treatment were taken as dependent variables, while the concentrations of root and leaf extracts (25, 50, 75, and 100%) and control/distilled water (0%) were considered independent variables.

To establish differences among the treatment means, the recorded data of the experiment were subjected to multivariate analysis of variance (MANOVA), which is an extension of analysis of variance. Multivariate analysis of variance (MANOVA) is used to compare groups of a number of different, but related, dependent variables, comparing the effects of different treatments and factors on a variety of outcome measures. Multivariate ANOVA can be used with one-way, two-way, and higher factorial designs involving one, two, or more independent variables. In this study, the dependent variables were the number of germinated seeds of *L. usitatissimum* and *G. abyssinica* (control and treatment groups or seeds treated with water and methanol extracts of *X. strumarium* leaf and root samples), plumule and radicle length, and seedling dry weight of both test plant species. Besides, the factors or independent variables were the concentration of water and methanol extracts of *X. strumarium* leaf and root samples at five levels (0/control, 25%, 50%, 75%, and 100% concentrations).

A multivariate analysis of variance was conducted to identify the effects of concentration differences of water and methanol extracts of *X. strumarium* leaves and roots samples in the set of dependent variables (number of germinated seeds, Plumule and radicle length, and seedling dry weight of *G. abyssinica* and *L. usitatissimum* seeds).

**Box's Test:** Box's Test of Equality of Covariance Matrices indicates whether the data violates the assumption of homogeneity of variance-covariance matrices. If the sig. value is greater than 0.001, the assumption is not violated.

**Levene's Test:** In this test, any values that are less than 0.05 would indicate that the assumption of equality of variance is violated for that variable.

**Multivariate tests** indicate whether there are statistically significant differences among the groups based on a linear combination of the dependent variables. There are a number of statistics to choose from (Wilks' Lambda, Hotelling's Trace, and Pillai's Trace). One of the most commonly reported statistics is Wilks' Lambda.

**Wilks' lambda** demonstrates the amount of variance accounted for in the dependent variable by the independent variable; the smaller the value, the larger the difference between

the groups being analyzed. 1 minus Wilks' lambda indicates the amount of variance in the dependent variables accounted for by the independent variables. In addition, if the significance level is less than 0.05, then it can be concluded that there is a difference among the dependent variables (Pallant, 2011).

When there are independent variables with three or more levels, it is necessary to conduct follow-up univariate analyses to identify where the significant differences lie. One way to do this would be to use a one-way ANOVA on the dependent variables that were significant in the MANOVA. A Tukey's Honestly Significant Difference (HSD) Test ($p \leq 0.05$) was employed for mean separations when the MANOVA revealed significant treatment differences. Besides, germination parameters were also calculated using the following equations:

### Germination Percentage (GP)

Germination percentage is the percentage of seeds in a container or lot that germinate under favorable germination conditions (Kader, 2005).

$$\text{Germination Percentage (GP)} = \left(\frac{Total\ number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sowed}\right) * 100$$

### Mean Germination Time (MGT)

Mean Germination Time is a precise measure of the period taken for the given seeds to germinate.

$$\text{Mean Germination Time (MGT)} = \left(\frac{\sum TiNi}{S}\right)$$

where Ti = number of days after the beginning of the trial, Ni = number of seeds germinated on the day, and S = total number of seeds germinated (Kader, 2005).

### Coefficient of Velocity of Germination (CVG)

CVG is a signal of the speediness of germination. It rises when the number of germinated seeds increases and the time required for germination drops.

$$\text{Coefficient of Velocity of Germination}(CVG) = \left(\frac{N1+N2i+\cdots+Nx}{N1T1+N2T2\ldots.+NxTx}\right)$$

where N = number of seeds germinated each day, T= number of days from seeding parallel to N (Kader,2005; Ranal and Santana,2006).

## 3. Results

### Effects of aqueous extracts of *X. strumarium* on the germination of test plant seeds

The outcome of this investigation confirmed that there were substantial distinctions in the mean numbers of *G. abyssinica* (L.f.) Cass. and *L. usitatissimum* L. seeds germinated per treatment. The quantities of seeds sprouted per treatment were concentration-dependent. Subsequently, as the concentration of water extracts from each sample increased, the number of seeds germinating per treatment declined. There were strong negative associations between water extracts of leaf and root samples of *X. strumarium* and the quantity of germinated seeds of *G. abyssinica,* as shown in the regression equation: $Y = -20.533X + 26.667$, $R^2 = 0.9552$ (Figure1a) and $Y = -11.2X + 26.267$, $R^2 = 0.8798$ (Figure 1b), respectively.

There was also a tough inverse linear association among the number of *L. usitatissimum* seeds sprouted and the concentration of water extracts from leaf and root samples of *X. strumarium,* as indicated in the regression equations Y = -22.933X + 26.4, $R^{2=}$ 0.9697 (Figure 1c) and Y = -14.4X + 27.067, $R^2$ = 0.8607 (Figure 1d) correspondingly. Generally, water extracts from leaf and root samples of *X. strumarium* significantly reduced the germination of the test plant species.

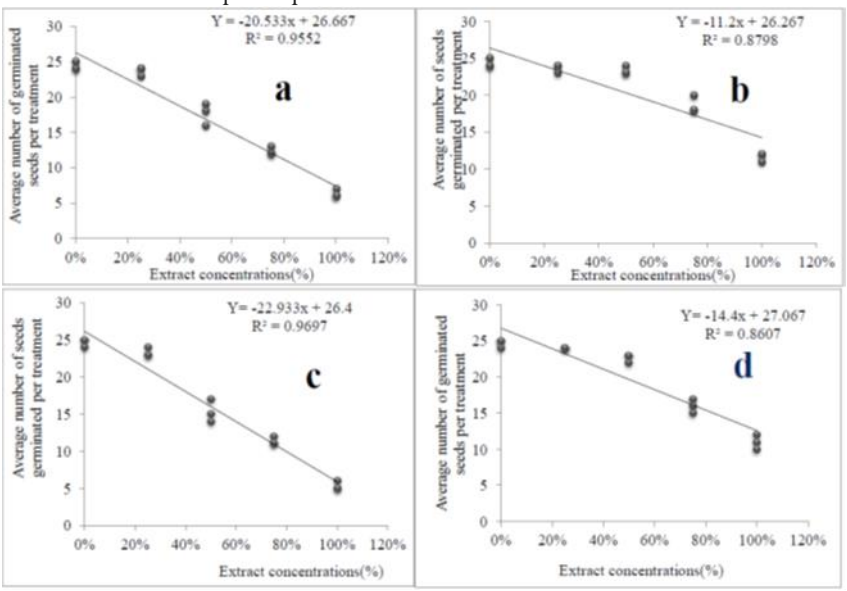


Figure 1. Effects of water extracts from leaf (a,c) and root (b,d) samples of *X. strumarium* on the germination of *G. abyssinica* (a,b) and *L. usitatissimum* (c, d), respectively.

As opposed to water extracts, in methanol extracts, the entire inhibition of germination was observed in both test plant species at ≥ 50% (treated with 50,75 and 100%) and ≥ 75% (75 and 100%) concentrations of leaf and root samples, respectively. Conversely, germination took place in both test plant species treated with methanol extracts of root at ≤ 50 % (tested with 25 and 50% concentrations) and leaf at ≤ 25% (25%) samples, respectively.

## Effects of aqueous extracts of *X. strumarium* on germination parameters

### Germination Percentage (GP)

The maximum mean germination percentage was 98.7% which recorded in the control group of *L. usitatissimum*, followed by 97.3% (*G. abyssinica*), while the minimum mean germination percentage was 21.3%, in *L. usitatissimum* seeds tested with 100% concentrations aqueous (water) extracts of *X. strumarium* leaf samples, followed by 25.3% (*G. abyssinica*). Maximum concentration (100%) of aqueous extracts (water) of leaf sample resulted in 72 and 77.4% inhibition of *Guizotia abyssinica* and *Linum usitatissimum* seeds germination, correspondingly compared to the control (Tables 3A1_A2 and B1_B2). *G. abyssinica* and *L. usitatissimum* seeds experimented with a 25% concentration of methanol extract from leaf samples had mean germination percentage of 32% and 28%, respectively. Furthermore, the germination percentage of *G. abyssinica* and *L. usitatissimum* seeds treated with *X. strumarium* root extract at 50% concentration was 24% and 20%, correspondingly (Figure 2).

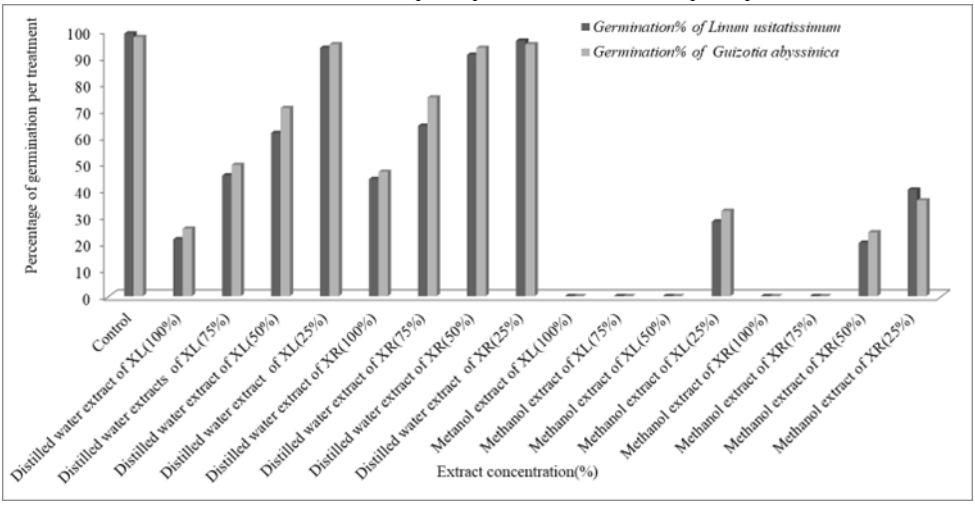


Figure 2. Effect of water and methanol extracts of leaf and root samples of *X. strumarium* on the GP of *G. abyssinica* and *L. usitatissimum* seeds, where XL*: X. strumarium* leaf extracts, *XR: X. strumarium* root extracts.

## Interpretation of output from Multivariate analysis of variance (MANOVA) for number germinated seeds per treatments

Initially, normality, linearity, outliers, homogeneity of variance covariance matrices, Box's Test of Equality of Covariance Matrices, Levene's, Wilks' Lambda, and multicollinearity tests for the number of germinated seeds of *G. abyssinica* and *L. usitatissimum* that were treated with water and methanol extracts of *X. strumarium* leaf and root samples were analyzed, and the results indicated that no serious violations occurred.

## Effect size

The significance impact of water and methanol extracts of *X. strumarium* leaf and root samples on the germination of *L. usitatissimum* and *G. abyssinica* seeds can be evaluated using the effect size statistic. Partial eta squared represents the proportion of the variance in the dependent variable (germinations of test seeds) that can be explained by the independent variable (concentrations of water and methanol extracts of roots and leaf samples). Partial eta squared values for the germination of *G. abyssinica* and *L. usitatissimum* seeds treated with water extracts of leaf samples were 0.987 and 0.991, respectively. These represent 98.7 and 99.1% of the variance in the germination of *G. abyssinica* and *L. usitatissimum* seeds, explained by the concentration of water extracts in leaf samples, respectively. Besides, the germination of *G. abyssinica* and *L. usitatissimum* seeds treated with methanol extracts of leaf samples was 0.996 and 0.989, respectively. Hence, these represent 99.6 and 98.9% of the variance in the germination of *G. abyssinica* and *L. usitatissimum* seeds explained by the concentration of methanol extracts in leaf samples. Partial eta squared values for the germination of *G. abyssinica* and *L. usitatissimum* seed treated with water extracts of root samples were 0.98 and 0.982. Besides, the germination of *G. abyssinica* and *L. usitatissimum* seeds treated with methanol extracts of root samples was 0.991 and 0.977, respectively. Generally, according to generally accepted criteria, the concentration of both water and methanol extracts in leaf and root samples had quite a large effect on the germination of both test plant species (Table 1).

Table 1. Tests of Between-Subjects Effects or germination of seeds treated with water and Methanol extracts of *X. strumarium* leaves and roots samples

| Source | Dependent Variable | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| Corrected Model | GerminationGWEL | 784.933[a] | 4 | 196.233 | 196.233 | .000 | .987 |
| | GerminationLWEL | 989.067[b] | 4 | 247.267 | 285.308 | .000 | .991 |
| | GerminationGMEL | 1336.000[c] | 4 | 334.000 | 626.250 | .000 | .996 |
| | GerminationLMEL | 1404.267[d] | 4 | 351.067 | 188.071 | .000 | .987 |
| | GerminationGWER | 354.400[e] | 4 | 88.600 | 120.818 | .000 | .980 |
| | GerminationLWER | 410.267[f] | 4 | 102.567 | 139.864 | .000 | .982 |
| | GerminationGMER | 1199.067[g] | 4 | 299.767 | 281.031 | .000 | .991 |
| | GermintionLMER | 1190.400[h] | 4 | 297.600 | 106.286 | .000 | .977 |
| Intercept | GerminationGWEL | 3969.067 | 1 | 3969.067 | 3969.067 | .000 | .997 |
| | GerminationLWEL | 3315.267 | 1 | 3315.267 | 3825.308 | .000 | .997 |
| | GerminationGMEL | 601.667 | 1 | 601.667 | 1128.125 | .000 | .991 |
| | GerminationLMEL | 721.067 | 1 | 721.067 | 386.286 | .000 | .975 |
| | GerminationGWER | 6080.267 | 1 | 6080.267 | 8291.273 | .000 | .999 |
| | GerminationLWER | 5762.400 | 1 | 5762.400 | 7857.818 | .000 | .999 |
| | GerminationGMER | 928.267 | 1 | 928.267 | 870.250 | .000 | .989 |
| | GermintionLMER | 912.600 | 1 | 912.600 | 325.929 | .000 | .970 |
| Concentration | GerminationGWEL | 784.933 | 4 | 196.233 | 196.233 | .000 | .987 |
| | GerminationLWEL | 989.067 | 4 | 247.267 | 285.308 | .000 | .991 |
| | GerminationGMEL | 1336.000 | 4 | 334.000 | 626.250 | .000 | .996 |
| | GerminationLMEL | 1404.267 | 4 | 351.067 | 188.071 | .000 | .987 |
| | GerminationGWER | 354.400 | 4 | 88.600 | 120.818 | .000 | .980 |
| | GerminationLWER | 410.267 | 4 | 102.567 | 139.864 | .000 | .982 |
| | GerminationGMER | 1199.067 | 4 | 299.767 | 281.031 | .000 | .991 |
| | GermintionLMER | 1190.400 | 4 | 297.600 | 106.286 | .000 | .977 |
| Error | GerminationGWEL | 10.000 | 10 | 1.000 | | | |
| | GerminationLWEL | 8.667 | 10 | .867 | | | |
| | GerminationGMEL | 5.333 | 10 | .533 | | | |
| | GerminationLMEL | 18.667 | 10 | 1.867 | | | |
| | GerminationGWER | 7.333 | 10 | .733 | | | |
| | GerminationLWER | 7.333 | 10 | .733 | | | |
| | GerminationGMER | 10.667 | 10 | 1.067 | | | |
| | GermintionLMER | 28.000 | 10 | 2.800 | | | |
| Total | GerminationGWEL | 4764.000 | 15 | | | | |
| | GerminationLWEL | 4313.000 | 15 | | | | |
| | GerminationGMEL | 1943.000 | 15 | | | | |
| | GerminationLMEL | 2144.000 | 15 | | | | |
| | GerminationGWER | 6442.000 | 15 | | | | |
| | GerminationLWER | 6180.000 | 15 | | | | |
| | GerminationGMER | 2138.000 | 15 | | | | |
| | GermintionLMER | 2131.000 | 15 | | | | |
| Corrected Total | GerminationGWEL | 794.933 | 14 | | | | |
| | GerminationLWEL | 997.733 | 14 | | | | |
| | GerminationGMEL | 1341.333 | 14 | | | | |
| | GerminationLMEL | 1422.933 | 14 | | | | |
| | GerminationGWER | 361.733 | 14 | | | | |
| | GerminationLWER | 417.600 | 14 | | | | |
| | GerminationGMER | 1209.733 | 14 | | | | |
| | GermintionLMER | 1218.400 | 14 | | | | |

a. R Squared = .987 (Adjusted R Squared = .982)

b. R Squared = .991 (Adjusted R Squared = .988)

c. R Squared = .996 (Adjusted R Squared = .994)

d. R Squared = .987 (Adjusted R Squared = .982)

**Note: Germination-GWEL** and **Germination-LWEL:-** germination (number of germinated seeds) of *G. abyssinica* and *L. usitatissimum* seeds treated with water extracts of *X. strumarium* leaf samples respectively

| | |
|---|---|
| e. R Squared = .980 (Adjusted R Squared = .972) | **Germination-GMEL** and **Germination-LMEL:** - germination of *G. abyssinica* and *L. usitatissimum* seeds treated with methanol extracts of *X. strumarium* leaf samples respectively |
| f. R Squared = .982 (Adjusted R Squared = .975) | |
| g. R Squared = .991 (Adjusted R Squared = .988) | **Germination-GWER** and **Germination-LWER:** - germination of *G. abyssinica* and *L. usitatissimum* seeds treated with water extracts of *X. strumarium* root samples respectively |
| h. R Squared = .977 (Adjusted R Squared = .968) | **Germination-GMER** and **Germination-LMER:** - germination of *G. abyssinica* and *L. usitatissimum* seeds treated with methanol extracts of *X. strumarium* root samples respectively |

Generally, there was a statistically significant difference between water and methanol extracts of leaf and root samples on the combined dependent variables, F (8, 13) = 48.18, p = 0.000; Wilks' Lambda = 0.00; partial eta squared = 0.98. Once the results for the dependent variables were considered separately, the only variance to reach statistical significance, using a Bonferroni adjusted alpha level of 0.00625, were, F (4, 10) = 196.23 for GerminationGWEL, 285.31 for GerminationLWEL , 120.82 for GerminationGWER, 139.86 for GerminationLWER, 626.25 for GerminationGMEL, 188.1for GerminationLMEL , 282.03 for GerminationGMER and 106.29 for GerminationLMER ,p = 0.000, with partial eta squared values = 0.987, 0.991, 0.980, 0.982, 0.996,0.989, 0.991 and .977 respectively (Table 1).

## Comparing group means

Water and methanol extracts of *X. strumarium* leaf and root samples had already significantly impacted the germination of test plant species, but we do not know who had the higher scores. Concerning the effects of water extracts on the germination of test seeds, *L. usitatissimum* seeds treated with water extracts of *X. strumarium* root samples at 25% concentration had the highest mean score or mean number of germinated seeds (23.67˜24), followed by 23.33˜23 (G. *abyssinica* seeds treated with water extracts of roots at 25% concentration). The lowest mean score was recorded in *L. usitatissimum* seeds treated with water extracts of *X. strumarium* leaf samples at 100% concentration (3.67˜4), followed by 6 (G. *abyssinica* seeds treated with water extract leaf samples at 100% concentration). As to the effects of methanol extracts, *L. usitatissimum* seeds treated with water extracts of *X. strumarium* root samples at 25% concentration had the highest mean score or mean number of germinated seeds (10), followed by G. *abyssinica* seeds treated with water extracts of roots at 25% concentration. Conversely, the lowest score was recorded in both test seeds treated with methanol extracts of *X. strumarium* leaf samples at 50, 75, and 100% concentration and root samples at 75 and 100% concentration (0).

## Follow-up analyses

Water extracts of *X. strumarium* leaf and root samples had a significant impact on the germination of both test plant species based on the outcome of a multivariate analysis of variance (MANOVA). When there are independent variables with three or more levels, it is necessary to conduct follow-up univariate analyses to identify where the significant differences lie. In the current study, the independent variables (concentration of water and methanol extracts of leaves and root samples) had five levels (0% or the control, 25%, 50%, 75%, and

100% concentrations). One way to do this would be to use a one-way ANOVA on the dependent variables that were significant in the MANOVA. Hence, within the one-way ANOVA procedure, post-hoc tests were conducted (Table 2).

Table 2. One-way ANOVA/ Univariate Tests/ on the effect of water extracts from *X. strumarium* leaf and root samples on number of germinated seeds per treatment of *L. usitatissimum* and *G. abyssinica* seeds.

| | Test plant species | Parameters | Treated with water extracts of | DF | F value | P value |
|---|---|---|---|---|---|---|
| | *L .usitatissimum* | number of germinated seeds | Leaf | 4 | 270.9 | ≤ 0.001 |
| | | | Root | 4 | 198.7 | ≤ 0.001 |
| | *G. abyssinica* | | Leaf | 4 | 239.5 | ≤ 0.001 |
| | | | Root | 4 | 160.3 | ≤ 0.001 |
| | Test plant species | Parameters | Treated with methanol extracts of | DF | F value | P value |
| | *L .usitatissimum* | number of germinated seeds | Leaf | 4 | 188.07 | ≤ 0.001 |
| | | | Root | 4 | 106.29 | ≤ 0.001 |
| | *G. abyssinica* | | Leaf | 4 | 626.25 | ≤ 0.001 |
| | | | Root | 4 | 281.03 | ≤ 0.001 |

A Tukey's Honestly Significant Difference (HSD) indicated that the number of germinated seeds of both test crop seeds which treated at 50, 75, and 100% concentrations of water extracts of *X. strumarium* leaf samples was significantly different ($P \leq 0.001$) from the control. Besides, the number of germinated seeds of test crop seeds that treated with water extract of root samples at only 75 and 100% concentrations was significantly different ($P \leq 0.001$) from the control, whereas the number of germinated seeds of *L. usitatissimum* seeds that treated with water extract of root samples at 50% concentration was significantly different ($P \leq 0.05$) from the control.

Concerning Tukey's HSD Test for Methanol Extracts, the number of germinated seeds of both test crop seeds treated at 25, 50, 75, and 100% concentrations of methanol extracts of *X. strumarium* root and leaf samples were significantly different ($P \leq 0.001$) from the control. Besides, the number of germinated seeds of both test crop seeds that were treated with methanol extract of leaf samples at only 25% concentrations was significantly different ($P \leq 0.001$) from 50, 75, and 100%, while the number of germinated seeds of both test seeds that were treated with methanol extract of leaf samples at 100% concentration was not significantly different from 50 and 75% concentrations'. On the other hand, the number of germinated seeds of both test crop seeds treated with methanol extract of root samples at 25% concentrations was significantly different ($P \leq 0.001$) from 75, 100%, and 50% ($P \leq 0.01$). In addition, the number of germinated seeds of test crop seeds treated with methanol extract of root samples at 100% concentrations was significantly different from 50% ($P \leq 0.001$) for *G. abyssinica* and 50% ($P \leq 0.01$) for *L. usitatissimu.* Conversely, the number of germinated seeds of both test crop seeds treated with methanol extract of root samples at 100% concentrations was not significantly different from 75% concentration (seeds treated with 75% methanol extract of root samples).

## Mean Germination Time (MGT)

Seeds of *G. abyssinica* and *L.usitatissimum* treated with maximum concentration (100%) of water extracts of *X. strumarium* leaf samples exhibited the MGT of 9.28 (the longest MGT) and 7.95 respectively. However, the minimum MGT was 3.38 (in *L. usitatissimum* seeds) followed by 3.41 (*G. abyssinica* seeds) which was recorded in the control group. The MGT of *Guizotia abyssinica* and *Linum usitatissimum* seeds experimented with maximum concentration (100%) of water extracts of leaf samples increased by 63.25 and 57.43% correspondingly, compared to the control (Tables 3A1_A2 and B1_B2).

## Coefficient of Velocity of Germination (CVG)

The control group of *L. usitatissimum* seeds had the greatest mean CVG (33.01), followed by *G. abyssinica* (32.47). In contrast, the lowest mean CVG (15.29) was recorded in *L. usitatissimum* seeds treated with 100% water extract of leaf samples stalked by *G. abyssinica* (16.33). The mean CVG of *L. usitatissimum* and *G. abyssinica* seeds tested with 100% concentrations of water extract from *X. strumarium's* leaf samples declined by 53.68% and 49.7%, respectively, compared to the control (Tables $3A_1$_$A_2$ and $B1_B_2$).

Table 3. Effects of water extracts of leaf and root samples of *X. strumarium* on GP, MGT, and CVG of *G. abyssinica* ($A_1$ and $A_2$) and *L. usitatissimum* ($B_1$ and $B_2$) seeds expressed as Mean ± standard error (SE), respectively.

| $A_1$. | Leaf extracts concentration (%) | GP | MGT | CVG |
|---|---|---|---|---|
| 1. | Control(0) | 97.33±1.3 | 3.41±0.02 | 32.47±0.2 |
| 2. | 25 | 94.67±1.3 | 3.83±0.5 | 29.5±2.9 |
| 3. | 50 | 70.67±3.5 | 5.5±0.3 | 28.64±0.8 |
| 4. | 75 | 49.33±1.3 | 6.2±0.1 | 20.86±0.1 |
| 5. | 100 | 25.33±1.2 | 9.28±0.1 | 16.33±0.1 |
| $A_2$. | Root extracts concentration (%) | GP | MGT | CVG |
| 1. | Control(0) | 97.33±1.3 | 3.41±0.0 | 32.47±0.2 |
| 2. | 25 | 94.67±1.3 | 3.54±0.2 | 30.86±0.5 |
| 3. | 50 | 93.33±1.3 | 3.86±0.1 | 29.94±0.6 |
| 4. | 75 | 74.67±1.3 | 4.24±0.1 | 24.11±0.5 |
| 5. | 100 | 46.67±3.5 | 7.81±0.2 | 18.2±0.2 |
| $B_1$. | Leaf extracts concentration (%) | GP | MGT | CVG |
| 1. | Control(0) | 98.67±1.3 | 3.38±0.0 | 33.01±0.2 |
| 2. | 25 | 93.3±1.3 | 3.64±0.1 | 30.79±0.7 |
| 3. | 50 | 61.33±3.5 | 4.55±0.5 | 27.5±1.6 |
| 4. | 75 | 45.33±2.3 | 5.64±0.2 | 20.53±0.4 |
| 5. | 100 | 21.3±1.3 | 7.94±0.1 | 15.29±0.0 |
| $B_2$. | Root extracts concentration (%) | GP | MGT | CVG |
| 1. | Control(0) | 98.67±1.3 | 3.38±0.0 | 33.01±0.3 |
| 2. | 25 | 96±0.0 | 3.55±0.1 | 31.46±1.1 |
| 3. | 50 | 90.67±1.3 | 3.69±0.1 | 30.82±0.4 |
| 4. | 75 | 64.0±2.3 | 4.05±0.1 | 28.42±0.2 |
| 5. | 100 | 44.0±2.3 | 7.29±0.1 | 20.39±0.8 |

## Interpretation of output from Multivariate analysis of variance (MANOVA) for Plumule and Radicle length (in cm) of the seedlings

A one-way between-groups multivariate analysis of variance was performed to investigate the difference in concentration of water extracts of *X. strumarium* leaf and root samples in the plumule and radicle of the test plant species. The dependent variables were plumule and radicle length of *G. abyssinica* and *L. usitatissimum* seeds that were treated with water extracts of *X. strumarium* leaf and root samples. The independent variable was the concentration of water extracts from leaf and root samples. Normality, linearity, univariate and multivariate outliers, homogeneity of variance covariance matrices, Box's Test Equality of Covariance Matrices, Levene's and Wilks' Lambda tests, and multicollinearity were checked. As a result, serious violations of these tests were not detected. There was a statistically significant difference in concentration of water extracts of leaf and root samples on the combined dependent variables, F (7, 232) = 17.93, p = 0.000, Wilks' Lambda = 0.016, partial eta squared = 0.65, and partial eta squared (Table 4 last column).

Table 4. Tests of Between-Subjects Effects Plumule and radicle length of test seeds treated with water extracts of *X. strumarium* leaves and roots samples

| Source | Dependent Variable | Type III Sum of Squares | df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| Corrected Model | PlumuleLGWEL | 495.442[a] | 4 | 123.861 | 109.482 | .000 | .862 |
| | PlumuleLLWEL | 258.928[b] | 4 | 64.732 | 87.431 | .000 | .833 |
| | PlumuleLGWER | 419.942[c] | 4 | 104.986 | 80.775 | .000 | .822 |
| | PlumuleLLWER | 247.622[d] | 4 | 61.905 | 94.875 | .000 | .844 |
| | RadicleLGWEL | 503.116[e] | 4 | 125.779 | 192.560 | .000 | .917 |
| | RadicleLLWEL | 258.928[b] | 4 | 64.732 | 87.431 | .000 | .833 |
| | RadicleLGWER | 329.946[f] | 4 | 82.487 | 136.588 | .000 | .886 |
| | RadicleLLWER | 76.769[g] | 4 | 19.192 | 10.859 | .000 | .383 |
| Intercept | PlumuleLGWEL | 3437.467 | 1 | 3437.467 | 3038.434 | .000 | .977 |
| | PlumuleLLWEL | 2322.970 | 1 | 2322.970 | 3137.534 | .000 | .978 |
| | PlumuleLGWER | 3630.337 | 1 | 3630.337 | 2793.140 | .000 | .976 |
| | PlumuleLLWER | 2664.120 | 1 | 2664.120 | 4082.984 | .000 | .983 |
| | RadicleLGWEL | 2593.668 | 1 | 2593.668 | 3970.739 | .000 | .983 |
| | RadicleLLWEL | 2322.970 | 1 | 2322.970 | 3137.534 | .000 | .978 |
| | RadicleLGWER | 2967.307 | 1 | 2967.307 | 4913.497 | .000 | .986 |
| | RadicleLLWER | 2720.318 | 1 | 2720.318 | 1539.123 | .000 | .956 |
| Concentrations | PlumuleLGWEL | 495.442 | 4 | 123.861 | 109.482 | .000 | .862 |
| | PlumuleLLWEL | 258.928 | 4 | 64.732 | 87.431 | .000 | .833 |
| | PlumuleLGWER | 419.942 | 4 | 104.986 | 80.775 | .000 | .822 |
| | PlumuleLLWER | 247.622 | 4 | 61.905 | 94.875 | .000 | .844 |
| | RadicleLGWEL | 503.116 | 4 | 125.779 | 192.560 | .000 | .917 |
| | RadicleLLWEL | 258.928 | 4 | 64.732 | 87.431 | .000 | .833 |
| | RadicleLGWER | 329.946 | 4 | 82.487 | 136.588 | .000 | .886 |
| | RadicleLLWER | 76.769 | 4 | 19.192 | 10.859 | .000 | .383 |
| Error | PlumuleLGWEL | 79.193 | 70 | 1.131 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | PlumuleLLWEL | 51.827 | 70 | .740 | | | |
| | PlumuleLGWER | 90.981 | 70 | 1.300 | | | |
| | PlumuleLLWER | 45.675 | 70 | .652 | | | |
| | RadicleLGWEL | 45.724 | 70 | .653 | | | |
| | RadicleLLWEL | 51.827 | 70 | .740 | | | |
| | RadicleLGWER | 42.274 | 70 | .604 | | | |
| | RadicleLLWER | 123.721 | 70 | 1.767 | | | |
| Total | PlumuleLGWEL | 574.635 | 74 | | | | |
| | PlumuleLLWEL | 310.755 | 74 | | | | |
| | PlumuleLGWER | 510.923 | 74 | | | | |
| | PlumuleLLWER | 293.296 | 74 | | | | |
| | RadicleLGWEL | 548.839 | 74 | | | | |
| | RadicleLLWEL | 310.755 | 74 | | | | |
| | RadicleLGWER | 372.220 | 74 | | | | |
| | RadicleLLWER | 200.490 | 74 | | | | |

**Note:**

a. R Squared = .862 (Adjusted R Squared = .854)
b. R Squared = .833 (Adjusted R Squared = .824)
c. R Squared = .822 (Adjusted R Squared = .812)
d. R Squared = .844 (Adjusted R Squared = .835)
e. R Squared = .917 (Adjusted R Squared = .912)
f. R Squared = .886 (Adjusted R Squared = .880)
g. R Squared = .383 (Adjusted R Squared = .348)

**PlumuleLGWEL** and **PlumuleLLWEL**:- Plumule lengths of *G. abyssinica* and *L. usitatissimum* seeds treated with water extracts of *X. strumarium* leaf samples respectively

**PlumuleLGWER** and **PlumuleLLWER**: :- Plumule lengths of *G. abyssinica* and *L. usitatissimum* seeds treated with water extracts of *X. strumarium* root samples respectively

**RadicleLGWEL** and **RadicleLLWEL**: Radice lengths of *G. abyssinica* and *L. usitatissimum* seeds treated with water extracts of *X. strumarium* leaf samples respectively

**RadicleLGWER** and *RadicleLLWER: Radice* lengths of *G. abyssinica* and *L. usitatissimum* seeds treated with water extracts of *X. strumarium* root samples respectively

When the results for the dependent variables were considered separately, the only difference to reach statistical significance, using a Bonferroni adjusted alpha level of 0.00625, were PlumuleLGWEL, PlumuleLLWEL, PlumuleLGWER, PlumuleLLWER, RadicleLGWEL, RadicleLLWEL, RadicleLGWER and RadicleLLWER, F (4, 70) = 109.482, 87.431, 80.775, 94.875, 192.560, 87.431, 136.588 and 10.859 respectively, p = 0.000.

Following this one-way ANOVA was conducted on the dependent variables that were significant in the MANOVA. Hence, within the one-way ANOVA procedure post-hoc tests were conducted. Water extracts from leaf and root samples of *X. strumariums'* had a significant impact on the plumule and radicle length of both test plant species based on the result of a one-way ANOVA (Table 5).

Results (Tukey's HSD Test) showed that seedling growth or the plumule length of *G. abyssinica* and *L. usitatissimum* seeds tested with aqueous (water) extract of 50, 75, and 100% concentrations of *X. strumarium* leaf samples was significantly different ($P \leq 0.001$) from the control. Besides, the mean plumule length of *G. abyssinica* seeds treated with water extracts of root samples at concentrations of 50, 75, and 100% varied significantly ($P \leq 0.001$) compared to the control group. Likewise, the plumule length of seeds (*L. usitatissimum)* tried with aqueous (water) extract of *X. strumarium* root samples at concentrations of 75 and 100% showed significant differences ($P \leq 0.001$) compared to the control group.

Table 5. One-way ANOVA/ Univariate Tests/ on the effect of water extracts from *X. strumarium's* leaf and root samples on the plumule and radicle length of the test plant seedlings.

| | Test plant species | Parameters | Treated with water extracts of | DF | F value | P value |
|---|---|---|---|---|---|---|
| 1. | *L .usitatissimum* | Plumule length | Leaf | 4 | 63.1 | ≤ 0.001 |
| | | | Root | 4 | 18.5 | ≤ 0.001 |
| | | Radicle length | Leaf | 4 | 51.4 | ≤ 0.001 |
| | | | Root | 4 | 47.9 | ≤ 0.001 |
| 2. | *G. abyssinica* | Plumule length | Leaf | 4 | 109.5 | ≤ 0.001 |
| | | | Root | 4 | 80.8 | ≤ 0.001 |
| | | Radicle length | Leaf | 4 | 18.9 | ≤ 0.001 |
| | | | Root | 4 | 16.6 | ≤ 0.001 |

The control group of seedlings had mean plumule lengths of 7.63 cm (*L. usitatissimum)* and 9.9cm (*G. abyssinica,* the tallest). Conversely, the minimum plumule length (2.77 cm) of the seedlings was recorded in seeds *(L. usitatissimum)* experimented with concentrations of 100% water extracts of *X. strumarium* leaf samples, followed by 2.80 cm (*G. abyssinica).* Hence, the highest (100%) concentration of water extracts of leaf samples resulted in a 71.74 and 63.69% reduction in plumule length of *Guizotia abyssinica* and *Linum usitatissimum*, correspondingly (Tables 8A1_A2 and B1_B2).

The outcomes (Tukey's HSD Test) indicated the radicle length of seedlings of *L. usitatissimum* and *G. abyssinica* experimented with water extracts of *X. strumarium* root samples at 75 and 100% concentrations were substantially different ($P \leq 0.001$) from the control. In addition, the radicle length of seedlings of *G. abyssinica* tested by aqueous (water) extracts of leaf samples at 75 and 100% concentrations and *L. usitatissimum* treated at 50, 75, and 100% concentrations was significantly different ($P \leq 0.001$) from the control.

*G. abyssinica* and *L. usitatissimum* seedlings in the control group exhibited mean radicle lengths of 8.2cm (the tallest) and 7.3 cm, respectively. The smallest mean radicle length was 1.65 cm, which was recorded in *G. abyssinica* (seeds treated with 100% concentrations of water extract from leaf samples), followed by 2.53cm (in *L. usitatissimum* seeds). As a result, as compared to the control, the mean radicle length of *G. abyssinica* and *L. usitatissimum* seeds treated with water extracts of 100% concentration of leaf samples were reduced by 79.9% and 65.34% correspondingly (Tables $8A_1$_$A_2$ and B1_$B_2$).

Regression analysis revealed a strong negative linear relationship between the mean plumule length of *G. abyssinica* seedlings and the concentration of water extract from *X. strumarium* leaf and root samples with regression equation: $Y = -0.0678X + 10.047$, $R^2 = 0.807$ (Figure 3a) and $Y = -0.0556X + 9.3873$, $R^2 = 0.6885$ (Figure 3b), respectively. The mean plumule length of *L. usitatissimum* seedlings showed a negative linear relationship with the concentration of water extract from leaf and root samples of *X. strumarium*, as shown by regression equations $Y = -0.0513X + 8.1327$, $R^2 = 0.7954$ (Figure 3c) and $Y = -0.0477X + 8.3433$, $R^2 = 0.7263$ (Figure 3d), respectively.

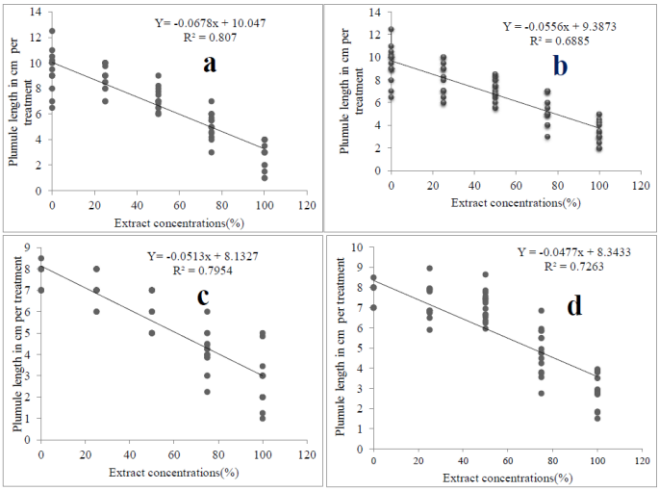


Figure 3. Effect of water extracts of leaf (a,c) and root (b,d) samples of *X. strumarium* on plumule length of *G. abyssinica* (a and b) and *L. usitatissimum* (c and d), respectively.

The concentration of water extracts from leaf and root samples and the mean radicle length had also a strong negative linear relationship. Accordingly, the regression equations for radicle length of *G. abyssinica* seedlings tested by water extract of leaf and root samples were: $Y = -0.063X + 7.4073$, $R^2 = 0.8711$ (Figure 4a) and $Y = -0.0565X + 7.2267$, $R^2 = 0.7151$ (Figure 4b), respectively. The regression equations for radicle length of *L. usitatissimum* seedlings that tested with water extract of leaf and root samples were: $Y = -0.0529X + 7.6457$, $R^2 = 0.773$ (Figure 4c) and $Y = -0.0278X + 7.4105$, $R^2 = 0.3603$ (Figure 4d), respectively.

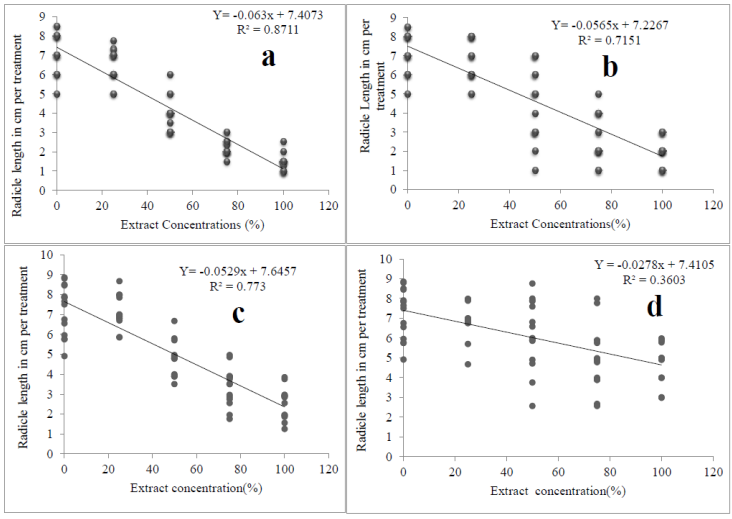


Figure 4. Effect of water extracts of leaf (a,c) and root (b,d) samples of *X. strumarium* on the radicle length of *G. abyssinica* (a and b) and *L. usitatissimum* (c and d), respectively.

## Seedling Dry Weight (mg)

### Interpretation of output from Multivariate analysis of variance (MANOVA) for seedlings dry weight (in mg)

A one-way between-groups multivariate analysis of variance was also conducted to investigate the effects of water extracts from leaf and root samples on the seedling dry weight of test plant species. First of all, normality, linearity, univariate and multivariate outliers, homogeneity of variance-covariance matrices, Levene's and Wilks' Lambda tests, and multicollinearity were checked. Consequently, no serious violations were detected. There was a statistically substantial variation between water extracts of leaves and roots samples on the joint dependent variables, F (16, 205) = 34.95, p = 0.000, Wilks' Lambda = 0.018, partial eta squared = 0.634, and individual partial eta squared values (Table 6 last column). Partial eta squared represents the proportion of the variance in the dependent variable (seedling dry weight) that can be explained by the independent variable (concentrations of water extracts from roots and leaf samples).

Table 6. Effects of water extracts of *X. strumarium* leaf and root samples on seedlings dry weight of test seeds (Tests of Between-Subjects)

| Source | Dependent Variable | Type III Sum of Squares | Df | Mean Square | F | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| Corrected Model | SDWLG | 1689.843[a] | 4 | 422.461 | 86.973 | .000 | .832 |
| | SDWLL | 1569.333[b] | 4 | 392.333 | 219.420 | .000 | .926 |
| | SDWRG | 960.469[c] | 4 | 240.117 | 60.336 | .000 | .775 |
| | SDWRL | 1291.996[d] | 4 | 322.999 | 107.873 | .000 | .860 |
| Intercept | SDWLG | 5877.728 | 1 | 5877.728 | 1210.061 | .000 | .945 |
| | SDWLL | 5682.842 | 1 | 5682.842 | 3178.238 | .000 | .978 |
| | SDWRG | 8699.683 | 1 | 8699.683 | 2186.044 | .000 | .969 |
| | SDWRL | 7649.538 | 1 | 7649.538 | 2554.730 | .000 | .973 |
| Concentrations | SDWLG | 1689.843 | 4 | 422.461 | 86.973 | .000 | .832 |
| | SDWLL | 1569.333 | 4 | 392.333 | 219.420 | .000 | .926 |
| | SDWRG | 960.469 | 4 | 240.117 | 60.336 | .000 | .775 |
| | SDWRL | 1291.996 | 4 | 322.999 | 107.873 | .000 | .860 |
| Error | SDWLG | 340.017 | 70 | 4.857 | | | |
| | SDWLL | 125.163 | 70 | 1.788 | | | |
| | SDWRG | 278.575 | 70 | 3.980 | | | |
| | SDWRL | 209.598 | 70 | 2.994 | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Total | SDWLG | 7907.588 | 75 | | | | |
| | SDWLL | 7377.338 | 75 | | | | |
| | SDWRG | 9938.728 | 75 | | | | |
| | SDWRL | 9151.133 | 75 | | | | |
| Corrected Total | SDWLG | 2029.859 | 74 | | | | |
| | SDWLL | 1694.496 | 74 | | | | |
| | SDWRG | 1239.045 | 74 | | | | |
| | SDWRL | 1501.595 | 74 | | | | |

a. R Squared = .832 (Adjusted R Squared = .823)

b. R Squared = .926 (Adjusted R Squared = .922)

c. R Squared = .775 (Adjusted R Squared = .762)

d. R Squared = .860 (Adjusted R Squared =

**Note:** SDWLG and SDWLL: seedling dry weights of *G. abyssinica* (a,b) and *L. usitatissimum* that trated with Water extracts of *X. strumarium* leaf samples SDWRG and SDWRL: : seedling dry weights of *G. abyssinica* (a,b) and *L. usitatissimum* that treated with Water extracts of *X. strumarium* rootsamples

While the results for the dependent variables were deliberated individually, the merely difference to reach statistical significance, using a Bonferroni adjusted alpha level of 0.0125, were seedlings dry weight of *G. abyssinica* and *L. usitatissimum* seeds that treated with water extracts of *X. strumarium* leaf and root samples, F (4, 70) = 86.97(SDWLG), 219.42(SDWLL), 60.34(SDWRG), and 107.87( SDWRL), p = 0.000,

Based on the result of a one-way ANOVA, water extracts of leaf and root samples of *X. strumarium* had a significant impact on the dry weight of seedlings of both test plant species (Table 7).

Table 7. One-way ANOVA/ Univariate Tests/ on the effect of water extracts from *X. strumarium* leaf and root samples on the dry weights of *L. usitatissimum* and *G. abyssinica* seedlings.

| Test plant species | Parameters | Extracts | DF | F value | P value |
|---|---|---|---|---|---|
| *L.usitatissimum* | Seedling dry weight | Leaf | 4 | 52.98 | ≤ 0.001 |
| | | Root | 4 | 27.95 | ≤ 0.001 |
| *G. abyssinica* | Seedling dry weight | Leaf | 4 | 51.84 | ≤ 0.001 |
| | | Root | 4 | 25.67 | ≤ 0.001 |

The seedling dehydrated weight of *L. usitatissimum* and *G. abyssinica* seeds experimented with water extract of leaf samples at 50, 75, and 100% concentrations was substantially different ($P \leq 0.001$) from the control, according to Tukey's Honestly Significant Difference (HSD)Test. Furthermore, the mean seedling dry weight of *G. abyssinica* and *L. usitatissimum* seeds treated with water extract of root samples from *X. strumarium* at 75 and 100% concentrations differed considerably ($P \leq 0.001$) from the control. On the other hand, the mean seedling dry weight of *L. usitatissimum* seeds treated with water extract of root samples at 50% concentration was substantially different ($P \leq 0.05$) from the control.

The maximum mean seedling dry weight (14.57 mg) was registered in the control group of *G. abyssinica,* followed by *L. usitatissimum* (control:14.13mg). The lowest mean seedling dehydrated weight was charted in seedlings of *G. abyssinica* (3.06 mg) (seeds tested with water extract of 100% concentration of *X. strumarium* leaf samples). Thus, *G. abyssinica* and *L. usitatissimum* seedling dry weight that was treated with water extracts of 100% concentration of leaf samples decreased by 78.99% and 78.2%, respectively, as compared to the control (Tables 8A1_A2 and B1_B2).

According to the regression analysis, the concentration of water extract from leaf and root sample of *X. strumarium* and seedlings dry weight of *G. abyssinica* showed negative linear relationship (Y = -0.1305X +15.379, $R^2$ = 0.787 (Figure 5a) and Y = -0.0958X + 15.559, $R^2$ = 0.694) (Figure 5b), respectively. There was also a negative linear relationship between the dry weight of the seedlings of *L. usitatissimum* and the concentration of water extract from leaf and root sample of *X. strumarium* (Y = -0.1259X + 14.998, $R^2$ = 0.8765 (Figure 5c) and Y = -0.1116X + 15.677, $R^2$ = 0.777 (Figure 5d), respectively).

Table 8. Effects of water extracts of leaf and root samples of *X. strumarium* on seedling growth of G. *abyssinica* ($A_1$ and $A_2$ and *L. usitatissimum* ($B_1$ and $B_2$), values expressed as Mean ± standard error (SE) respectively.

| $A_1$. | Leaf extracts concentration (%) | Plumule length(cm) | Radicle length(cm) | Dry seedling weight(mg) |
|---|---|---|---|---|
| 1. | Control(0) | 9.91±0.3 | 8.2±0.24 | 14.57±0.72 |
| 2. | 25 | 8.91±0.3 | 7.84±0.18 | 13.87±0.62 |
| 3. | 50 | 7.09±0.2 | 7.65±0.25 | 8.51±0.59 |
| 4. | 75 | 5.13±0.3 | 4.1±0.21 | 4.25±0.46 |
| 5. | 100 | 2.8±0.2 | 1.65±0.14 | 3.06±0.40 |
| $A_2$ | Root extracts concentration (%) | Plumule length(cm) | Radicle length(cm) | Dry seedling weight(mg) |
| 1. | Control(0) | 9.91±0.3 | 8.2±0.2 | 14.57±0.7 |
| 2. | 25 | 8.92±0.3 | 7.89±0.2 | 13.25±0.4 |
| 3. | 50 | 7.2±0.2 | 7.78±0.1 | 12.97±0.3 |
| 4. | 75 | 5.43±0.3 | 4.5±0.1 | 7.64±0.5 |
| 5. | 100 | 3.35±0.2 | 3.1±0.2 | 5.41±0.4 |
| $B_1$. | Leaf extracts concentration (%) | Plumule length(cm) | Radicle length(cm) | Dry seedling weight(mg) |
| 1. | Control(0) | 7.63±0.1 | 7.3±0.3 | 14.13±0.4 |
| 2. | 25 | 7.2±0.1 | 7.13±0.2 | 13.6±0.5 |
| 3. | 50 | 6.13±0.2 | 4.69±0.2 | 8.4±0.3 |
| 4. | 75 | 4.09±0.2 | 3.39±0.2 | 4.39±0.2 |
| 5. | 100 | 2.77±0.3 | 2.53±0.2 | 3.08±0.3 |
| $B_2$ | Root extracts Concentration (%) | Plumule length(cm) | Radicle length(cm) | Dry seedling weight(mg) |
| 1. | Control(0) | 7.63±0.14 | 7.3±0.32 | 14.13±0.36 |
| 2. | 25 | 7.31±0.21 | 6.9±0.23 | 13.66±0.62 |
| 3. | 50 | 7.11±0.19 | 6.2±0.44 | 12.15±0.42 |
| 4. | 75 | 4.84±0.28 | 4.9±0.41 | 7.07±0.42 |
| 5. | 100 | 2.91±0.19 | 4.8±0.26 | 3.48±0.36 |

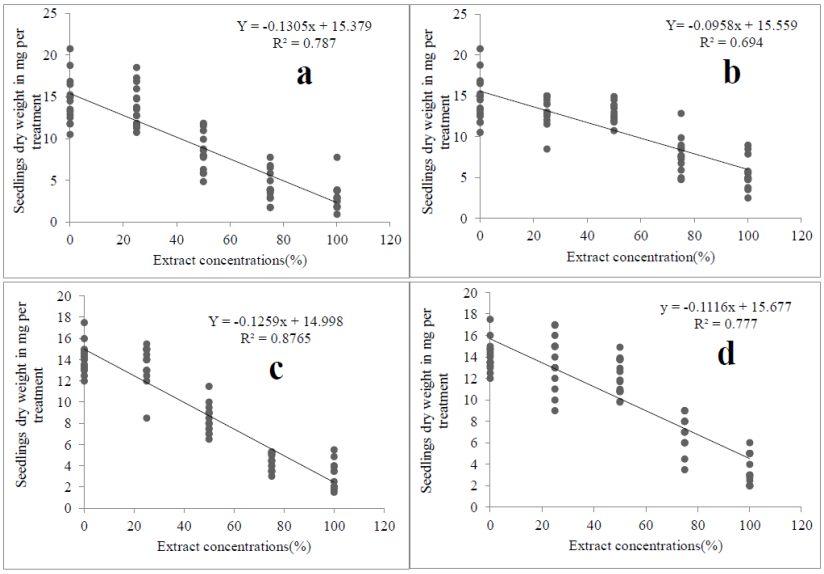


Figure 5. Effects of water extracts from leaf (a,c) and root (b,d) samples on the mean seedling dry weights of *G. abyssinica* (a,b) and *L. usitatissimum* (c, d), respectively.

# 4. Discussion

The results of the study indicated that aqueous extracts from leaf and root samples of *X. strumarium* significantly reduced the seedling growth and germination of *G. abyssinica* and *L. usitatissimum*. GP, CVG, plumule and radicle length, and dry weight of seedlings were reduced as concentrations of water extract from root and leaf samples increased compared to the control treatment. In agreement with the findings of our study, the result of the study by Benyas *et al.* (2010) indicated that aqueous extracts of shoot samples of *X. strumarium* affected the rate of germination, plumule length, and seedling dry weight of *Lens culinaris*. In line with the findings of our investigation, the result of the study by Kadioglu (2004) also indicated that *X. strumarium* inhibited the germination of *Triticum vulgare*, *Hordeum vulgare*, and *Avena sterilis* substantially.

In agreement with the findings of our study, the results of other studies also indicated that aqueous extracts of *X. strumarium* from different plant parts decreased germination, seedling growth, and the dry weight of *Pennisetum americanum, Lactuca sativa,* and *Brassica compestris* (Chon and Nelson, 2010). Moreover, In line with the findings of our study, the results of the study by Jalali *et al. (2013)* also showed that the germination of seeds and growth of *Zea maize* declined at higher concentrations of aqueous extracts of *X.strumarium,*. In agreement with our study, many other allelopathic studies of plant species confirmed that plants with allelopathic activity suppressed seedling height and the dry weight of receiver plants (Pisula and Meiners,2010; Tanveer *et al.*,2010; Zhang and Fu,2010; Favaretto *et al.*,2011; Fateh *et al.*,2012; Aguilera *et al.*,2015; Kapoor *et al.*,2019; Sodaeizadeh *et al.*,2019; Thiébaut *et al.*,2019, Seifu *et al.*,2023).

Moreover, the findings of this investigation indicated that as the concentration of water extracts from root and leaf samples of *X. strumarium* increased, CVG and GP decreased.

CVG gives an indication of the rapidity of germination. It rises when the number of germinated seeds increases and the time required for germination declines (Kader, 2005; Ranal and Santana, 2006; McNair *et al*., 2012; Sukifto *et al*., 2020). MGT is an exact measure of the period taken for the given seeds to sprout, but it does not correlate with the rate of germination (Kader, 2005). The result of this study showed that as the concentration of water extract from root and leaf samples of *X. strumarium* increased, the MGT also increased. The findings of our study are also in agreement with the outcomes of the investigation by Moosavi *et al*. (2011).

Higher concentrations of methanol extract in leaf ( ≥ 50%) and root (≥ 75%) samples of *X. strumarium* inhibited 100% seed germination of the test plant species, probably because methanol has a polar oxygen-hydrogen bond and a non-polar hydrocarbon chain that enable the extraction of both polar and non-polar allelochemicals from the specified samples. Methanol extracts did not totally inhibit germination at 25% (leaf) and 25% and 50% (root) concentrations. This might be the reduction of allelochemicals because of the dilution processes. Our finding is in agreement with that of Mehrafarin *et al*. (2011) and Seifu *et al*.(2023). In addition, in line with our finding of the study, Sitthinoi et al. (2017) also reported that methanol extract was severely inhibited the germination of seeds in two rice cultivars.

The inhibitory effect was more prominent with water extract of leaf than root samples of *X. strumarium*. GP, plumule, and radicle length were inhibited by 77.4, 63.69, and 65.3%, respectively, when *L. usitatissimum* seeds were tested with 100% concentrations of water extracts of leaf samples. Further, when *G. abyssinica* seeds were treated with 100% concentrations of water extract from leaf samples GP, radicle, and plumule length were inhibited by 72, 79.9, and 71.7%, respectively. In line with our investigation, the results of the study by El-Gawad *et al. (2019)* also indicated GP, root, and shoot growth were inhibited by 97.34, 98.45, and 93.56%, respectively, when *Bidens pilosa* seeds treated with *X. strumarium* essential oils.

# 5. Conclusions

The outcomes of this research demonstrated that the inhibitory impact of water and methanol extracts derived from both the root and leaf samples of *X. strumarium* varied depending on the concentration. As the concentrations of the extracts increased, the rate of inhibition also increased. Notably, the aqueous extracts from the leaf samples exhibited a stronger inhibitory effect compared to the root extracts. These findings suggest that the extracts obtained from this plant possess herbicidal properties. Consequently, further research involving phytochemical and molecular investigations is recommended to identify and isolate the active components present in *X. strumarium.* Moreover, exploiting isolated allelopathic substances as a means of controlling IAPS is suggested as an environmentally friendly approach to mitigate the deterioration of ecosystem Services.

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## Conflict of interests

The authors declare that they have no competing interests.

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