

*Full Length Research Paper*

# **Allelopathy as a tool for invasiveness of *Tithonia diversifolia* extracts through *in vitro* suppression of crop seeds' germination**

**Musabayana Zivanayi<sup>1\*</sup>, Mandumbu Ronald<sup>2</sup> and Mapope Nyamande<sup>1</sup>**

<sup>1</sup>Department of Crop Science, Marondera University of Agricultural Sciences and Technology, P. O. Box 35, Marondera, Zimbabwe.

<sup>2</sup>Department of Crop Science, Bindura University of Science Education, 741 Chimurenga Road, Off Trojan Road, Bindura, Zimbabwe.

Received 14 May, 2024; Accepted 14 June, 2024

**Wild sunflower (*Tithonia diversifolia*) is an invasive weed in both tropical and subtropical cultivated lands and propagates through seeds and plant cuttings. Invasive plants have distinctive functional traits that enable their invasiveness. The objective of this study was to determine the *in vitro* suppression of germination of crop seeds by extracts of *T. diversifolia*. Two experiments were carried out, one in the laboratory and the other in the greenhouse. The laboratory experiment was set up in a completely randomized design, while the greenhouse experiment was set up in a Randomized Complete Block Design. The blocking factor for the greenhouse experiment was the distance from the greenhouse door. Both experiments were replicated five times. *T. diversifolia* parts (leaves, stem, roots, and whole plant) were used in this experiment. The Genstat statistical package was used to analyze the data. Results showed that whole plant, leaf, stem, and roots extracts significantly ( $p < 0.001$ ) inhibited germination, radicle, plumule, stem girth extension, and chlorophyll content of maize, sunflower, soybeans, sorghum, rapoko grass, and cowpeas. This study confirmed that *T. diversifolia* parts suppressed germination, radicle, plumule, stem girth extension, and chlorophyll content of maize, sunflower, soybeans, rapoko grass, and cowpeas. Therefore, allelopathy contributes to the invasiveness of the weed.**

**Key words:** Growth inhibition, invasive, traits, colonize.

## **INTRODUCTION**

Pinpointing intrinsic functional traits associated with invasiveness to profile successful invaders has been a long-lasting quest in invasion ecology (Carboni et al., 2016). Traditionally, invasion success was studied by focusing on either the intrinsic biological characteristics

conferring invasiveness or characteristics of the resident community favoring invasion (Pyšek and Richardson, 2007). In Zimbabwe, information on the invasion biology and ecological consequences of *Tithonia diversifolia* is scanty, Regardless of the weed occupying both natural

\*Corresponding author. E-mail: [musabayana77@gmail.com](mailto:musabayana77@gmail.com).

and agricultural ecosystems (Chukwuka et al., 2014). *T. diversifolia* is an aggressive colonizer which competes with crops for resources, reduces farm and forest productivity, invades crops, smothers pastures, and in some cases can harm livestock. It poses a significant threat to the integrity and biodiversity of native systems; hence, farmers are facing tremendous challenges in its control (Ronald et al., 2011). Research by Gharde et al. (2018) in India shows that globally, weeds are responsible for decreasing the production of the world's eight most important food and cash crops by 13.2%. The author further stated that more than USD 100 billion in economic losses are incurred worldwide, and USD 25 billion is spent on buying herbicides annually. In Central and Eastern Europe, 60,000 hectares or 10% of all croplands were abandoned between 1990 and 2004 in Hungary (Valkó et al., 2016). Abandoned croplands are usually sensitive to the immigration of an invasive species such as *T. diversifolia*. In Asia and the Pacific region, 578 million people go hungry due to the effects of invasive weeds (Yaduraju and Rao, 2013). In Africa, estimated losses of US \$200 million per year that increase annually by US \$30 million in rice yield are caused by parasitic weeds (Rodenburg et al., 2016). The widespread and yield-depressing nature of invasive weeds therefore threatens the livelihood of about 300 million farmers in 44 countries, resulting in revenue loss of about 4 billion US dollars annually (Olakojo, 2004). In Zimbabwe, more than 75% of time is spent in controlling invasive weeds in the peak period of November and February (Ronald et al., 2011).

Weeds consist of plants that may be annual, biennial, or perennial as well as perennial vines, shrubs, and trees (Muoghalu and Chuba, 2005). A large proportion of these invasive plants, including *T. diversifolia*, are terrestrial species that require sunny habitats, while others are adapted to varying degrees of lower light levels under thickets or forest canopies. Invasive weeds are present in most ecosystems on earth, including water, roadsides, wetlands, and in elevated hot deserts in the United States (Perkins and Nowak, 2013) and globally. In Zimbabwe, weeds in agro-ecological zones are abundant, and most of them are invasive. Although *T. diversifolia* has been identified in most agro-ecological zones, each has different types of weeds due to different environmental conditions and soil types. *T. diversifolia* has been present in South Africa since 1946, while the first herbarium specimen collected in Zimbabwe was in 1944 near Bulawayo (Shackleton et al., 2019). It was introduced in Zimbabwe as an ornamental (Rejmánek and Richardson 2013); subsequently, it became invasive, threatening biodiversity and agricultural areas. The weed was first seen in Zambia in the 1950s, with the first record for eastern Africa from Zanzibar in 1949 (Arne, 2019). *T. diversifolia* is regarded as being invasive in Nigeria (Otusanya and Ilori, 2014). In southern Africa, *T. diversifolia* is now widespread in Malawi, Zambia, and

Zimbabwe (Shackleton et al., 2019). It has not been recorded in Angola (Rejmánek et al., 2017). The weed is rare in Botswana, Mozambique, and Namibia and uncommon in Kenya, Swaziland, Tanzania, and Uganda with one record for Ethiopia (Shackleton et al., 2019). Wild sunflower (*T. diversifolia*) is one of the problematic invasive weeds in Zimbabwe, where it is a generalist, capable of surviving in a variety of different locations, habitats, and conditions. Despite some studies conducted by Shackleton et al. (2019) in Zambia, no information is available about *T. diversifolia* in Zimbabwe, yet it is found occupying various habitats.

Weeds do not exhibit similar characteristics in terms of their invasiveness. *Tithonia* spp. possess allelochemicals, as demonstrated in a study by Musyimi et al. (2012) in Kenya, which indicated that *T. diversifolia* inhibits the growth of cowpeas. A study by Chukwuka et al. (2014) shows that *T. diversifolia* has both growth-inhibiting and growth-stimulating properties. There is no literature about the allelopathic effect of *T. diversifolia* on other crops. Hence, the objective is to study the allelopathic effects of the weed on maize, sunflower, soya beans, sorghum, rapoko grass, and cowpeas in Zimbabwe. Numerous studies about allelopathic effects, dormancy, and dispersal of other invasive weeds have been conducted; however, no research has been conducted about the wild sunflower (*T. diversifolia*). Due to different climatic conditions between Zimbabwe and Kenya, where Musyimi et al. (2012) conducted research on *T. diversifolia*, which is the most abundant and a problem weed in Zimbabwe. This research will investigate the traits that are contributing to its weediness in Zimbabwe and determine which herbicide has the greatest efficacy for its control. Although there are numerous herbicides on the market, no control measure can currently be recommended for the control of *T. diversifolia* in Zimbabwe. Given that *Tithonia* spp. are believed to release allelochemicals, and its seeds show long persistence in the soil and are easily spread, there is a need to study how these influence *T. diversifolia* interference, longevity, and colonization in Zimbabwean agro-systems. The objective of the study is to determine the allelopathic effects of different parts of *T. diversifolia* on crop growth of field crops *in-vitro*.

## MATERIALS AND METHODS

### Experimental site

A bioassay of maize, soybean, sunflower, sorghum, rapoko grass, and cowpea was conducted in the laboratory at Kushinga Phikelela Polytechnic College, Mashonaland East Province, Marondera District, Zimbabwe, during the 2021/2022 and 2022/2023 seasons. The location lies within coordinates (S18°15'127" and E316°68'163") on the global positioning system (GPS) and has an altitude of 1400m above sea level. It falls under natural ecological region IIb in Zimbabwe, characterized by a unimodal rainfall pattern with 850 mm during the cropping season of 2020. The soil type is sandy loam with a pH of 5.8, organic carbon content of 2%, total

**Table 1.** Treatments obtained from *T. diversifolia*.

Treatment number	<i>T. diversifolia</i>	Notes
1	Control water	Distilled water
2	Roots	Root extracts
3	Leaves	Leaves extracts
4	Stems	Stems extracts
5	Whole plant	Whole plant extracts

nitrogen of 0.17%, Bray P1 of 55.7 ppm, exchangeable acidity of 1.28, cation exchange capacity (CEC) of 4.14 meq/100 g, and magnesium of 0.6 meq/100 g.

### Plant

The plant material used in the study consisted of *T. diversifolia* parts (leaves, roots, stem) and the whole plant, as well as crop seeds from maize, soybeans, sunflower, sorghum, rapoko grass, and cowpeas.

### Laboratory assay

#### Experimental design/treatments

The crop factor bioassay was laid out in a Completely Randomized Design. The main factors were the roots, leaves, stems, and whole plant extracts of *T. diversifolia*, plus a control (distilled water). The sub-plots were the test plants (maize, soybeans, sunflower, sorghum, rapoko grass, and cowpeas). The treatments were replicated five times.

#### Treatment preparation

Healthy, fresh stocks of *T. diversifolia*, about 2 kg each, at the flowering stage were pulled randomly from infested fields. The weed was taken to the laboratory and separated into leaves, roots, stems, and whole plant extracts. Extracts of the *T. diversifolia* weed parts (leaves, stems, and roots) at a concentration of 100% were prepared by crushing 500 g of each part (roots, leaves, and stems) and the whole plant separately with a pestle and mortar. Two liters of water were added to each treatment. The solutions were stirred for 24 h at room temperature (25°C) on an orbital shaker at 100 rpm. The collected extracts were filtered through cheesecloth to remove debris and finally filtered using Whatman No. 1 filter paper to achieve a 100% concentration according to Musyimi et al. (2012). To ascertain the inhibitory activity exhibited by different parts of *T. diversifolia*, germination bioassays were conducted. The Petri dish germination bioassay was done in a laboratory where all conditions were controlled, with the temperature kept at 25°C. The treatments were used in subsequent experiments. Pot experiments were conducted to substantiate the results. Table 1 shows the treatments obtained from *T. diversifolia*.

#### Experimental management

The test units used were Petri® dishes with a diameter of 9 cm and a depth of 3 cm. To prevent the growth and activity of variant microbes, the Petri dishes were disinfected by cleaning them with formalin. Fifteen seeds of each test crop (crop factor) obtained from

Farm and City shop in Harare, Zimbabwe, were sterilized using 1% sodium hypochlorite for 10 minutes, then rinsed four times with distilled water, and placed in Petri® dishes lined with Whatman No. 2 filter paper. The seeds in the Petri® dishes were treated with 10 ml of the respective extract concentration, sealed with foil paper, and laid out in a Completely Randomized Design as described above. To neutralize evaporation and changes to the various extracts, the caps of the Petri® dishes were closed firmly. Five plots were laid out in a Completely Randomized Design, each plot containing five treatments, which were replicated five times for a total of twenty-five Petri® dishes per plot. The test plants were randomly allocated to the main plots and replicated five times.

#### Data collection

Data on germination and radicle length was collected from five randomly selected plants on the 8th, 10th, 12th, and 14th days after germination. Germination was considered to have occurred when the radicle was at least 2 mm long (Rugare, 2018). The length of the radicle was measured using a transparent meter ruler up to the end of the experiment. Germination percentage (G%) was computed using the following formula (1):

$$G\% = (\text{Number germinated} / \text{Number planted}) \times 100 \quad (1)$$

The length of the radicle and plumule of each test crop were measured in centimetres daily, from the day of germination and up to four days using a ruler.

#### Statistical analysis

Analysis of variance (ANOVA) of data on germination and radicle length of maize, soybean, sunflower, and cowpeas was performed using the Genstat statistical package (VSN International Limited). The standard error of the difference was used to separate treatment means at the 5% level of significance.

### Greenhouse assay

#### Experimental design and management

A bioassay experiment was set up in pots using a split plot design at Grasslands Research Institute in Marondera. Four treatments were extracted from *T. diversifolia* leaves, stems, roots, and the whole plant, with distilled water used as the control. The treatments were replicated five times. The test units used were pots filled with sandy loam soil and laid in an open space. The pots were perforated at the bottom to avoid waterlogging. Fifteen seeds of each crop species were sown in the pots, with each treatment replicated five times. Watering was done every morning with 100%

**Table 2.** Effects of aqueous extracts on seeds germination in the laboratory assay.

Treatment	Cow peas	Maize	Soya beans	Sunflower	Sorghum	Rapoko grass
Control	83.50 <sup>a</sup>	92.50 <sup>a</sup>	91.90 <sup>a</sup>	85.70 <sup>a</sup>	91.30 <sup>a</sup>	60.30 <sup>a</sup>
Root	27.60 <sup>b</sup>	33.00 <sup>b</sup>	64.80 <sup>b</sup>	50.40 <sup>b</sup>	2.980 <sup>b</sup>	17.10 <sup>b</sup>
Stem	22.70 <sup>b</sup>	10.00 <sup>c</sup>	18.50 <sup>c</sup>	10.00 <sup>c</sup>	1.000 <sup>c</sup>	10.00 <sup>b</sup>
Leaf	10.00 <sup>b</sup>	10.00 <sup>c</sup>	10.00 <sup>c</sup>	10.00 <sup>c</sup>	1.000 <sup>c</sup>	10.00 <sup>b</sup>
Whole plant	10.00 <sup>b</sup>	10.00 <sup>c</sup>	10.00 <sup>c</sup>	10.00 <sup>c</sup>	1.000 <sup>c</sup>	10.00 <sup>b</sup>
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by different letters are significantly different at the 0.01 probability level.

*T. diversifolia* extracts per pot for two weeks.

#### Data collection

Data on germination and plumule length were collected from five randomly selected plants soon after germination on the 14th, 15th, 16th, and 17th days. Germination was considered to have occurred when the radicle reached 2 mm in length (Rugare, 2018). The length of the plumule of each test crop species was measured in centimeters on the 14, 15, 16, and 17th days after germination using a ruler. Data on chlorophyll content, leaf biomass, and root biomass were collected one month after planting.

#### Statistical analysis

Analysis of variance (ANOVA) of data on germination, plumule length, chlorophyll content, stem girth, and leaf and root biomass of the crop species was performed using the Genstat statistical package (VSN International Limited). Data were tested for normality using the Shapiro–Wilk test. The standard error of the difference was used to separate treatment means at the 5% level of significance.

## RESULTS

The results of the effects of *T. diversifolia* extracts are presented in Table 2. Analysis of variance indicated a significant difference ( $p < 0.05$ ) in the germination of crop seeds in the laboratory following the application of treatments. The control exhibited the highest germination percentage among all treatments.

Leaf and whole plant extracts showed the highest inhibition, severely reducing germination across all tested crops. The presence of *T. diversifolia* significantly reduces germination, promoting its dominance in habitats where it is the primary occupant alongside a few resistant species.

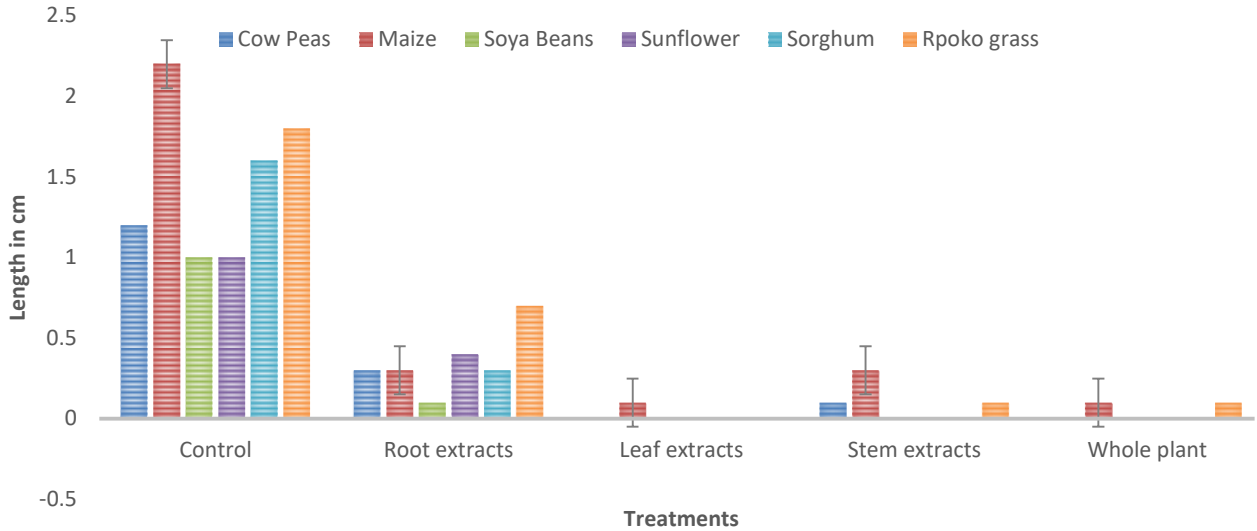
Stem extracts notably inhibited all treated plants compared to the control, particularly affecting maize, sunflower, sorghum, and rapoko grass. Root extract had the most significant impact on sorghum germination, with only 3% germination observed, and to a lesser extent on rapoko grass. There was no significant difference ( $p < 0.05$ ) in the germination of sunflower and cowpeas after treatment with root extracts. Very low germination rates

were observed across all treatments treated with leaf and whole plant aqueous extracts. Germination of maize, sunflower, sorghum, and rapoko grass was notably low in stem extract treatments. The detrimental effects of *T. diversifolia* on crop seed germination have been clearly demonstrated (Table 2). The effects of *T. diversifolia* aqueous extracts on radicle length on day 8 in the laboratory assay are depicted in Figure 1.

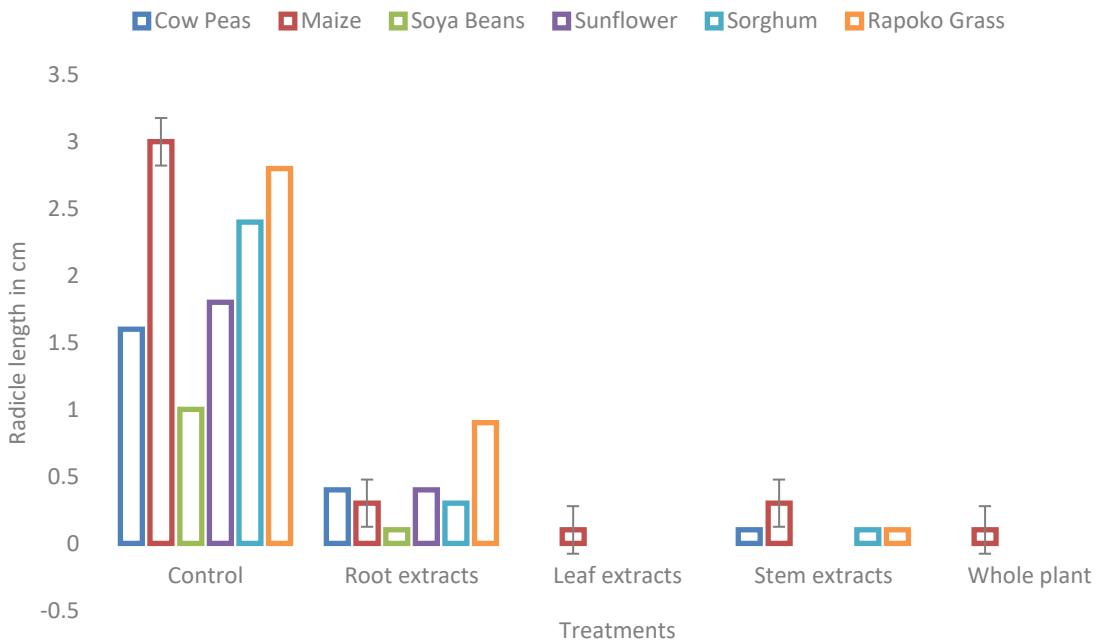
On day 8, a significant difference ( $p < 0.05$ ) was observed between the control and aqueous extracts from different plant parts on the length of the radicle of all crop species. The negative impact of *T. diversifolia* aqueous extracts on the radicle growth of crop species has been established. The control exhibited the greatest radicle growth in all crops.

Whole plant extracts and leaf extracts showed the most pronounced inhibitory effects on radicle growth across all crop species, followed by stem and root extracts in the laboratory (Figure 1). There was no significant difference ( $p > 0.05$ ) among whole plant, leaf, and stem extracts in their effects on radicle growth of all crop species on day 8. Radicle growth was consistently reduced in all *T. diversifolia* aqueous extracts by day 8. The primary allelopathic effects include delayed radicle growth, which subsequently leads to physiological impacts that reduce growth and dry matter accumulation in plantlets. The effects of extract treatments on radicle length on day 9 are illustrated in Figure 2.

On day 9, a significant difference ( $p < 0.05$ ) in radicle growth was observed among all treatments. The control exhibited the greatest radicle length, followed by root extracts. The radicle length of germinated seedlings was reduced by the extracts, confirming the presence of mitotic inhibitors within the complex of allelochemicals in *T. diversifolia* extracts. The greatest inhibition was observed in soybeans among all treatments. Rapoko grass was less affected by root extracts compared to the control, although its radicle growth was still lower. Leaf extracts exerted the most pronounced inhibitory effects on all tested crops except maize, where slight radicle growth was observed. Leaf, stem, and whole plant extracts showed significant inhibitory effects across all crops (Figure 2). The effects of *T. diversifolia* aqueous extracts on radicle length on day 10 in the laboratory



**Figure 1.** Length of the radicle on the 8th day after treatment with *T. diversifolia* extracts.



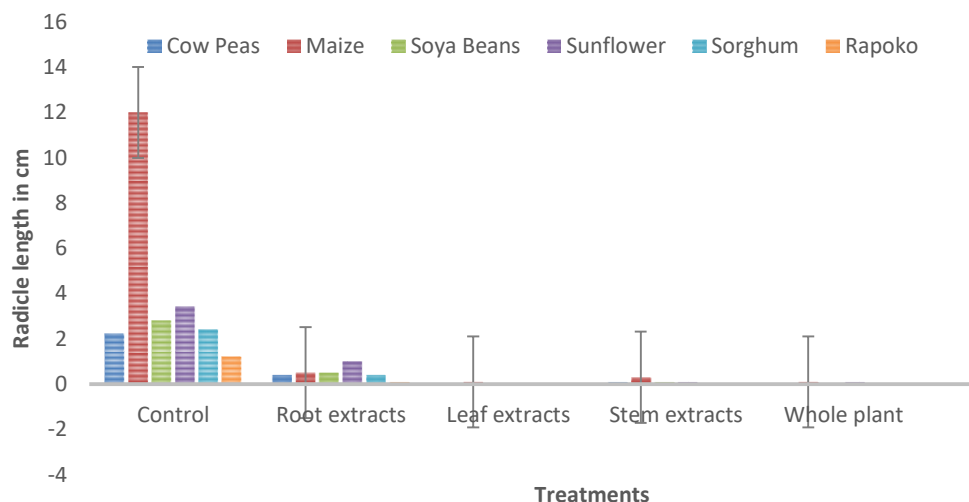
**Figure 2.** Length of radicle on day 9 after treatment with the *T. diversifolia* extracts.

assay are depicted in Figure 3.

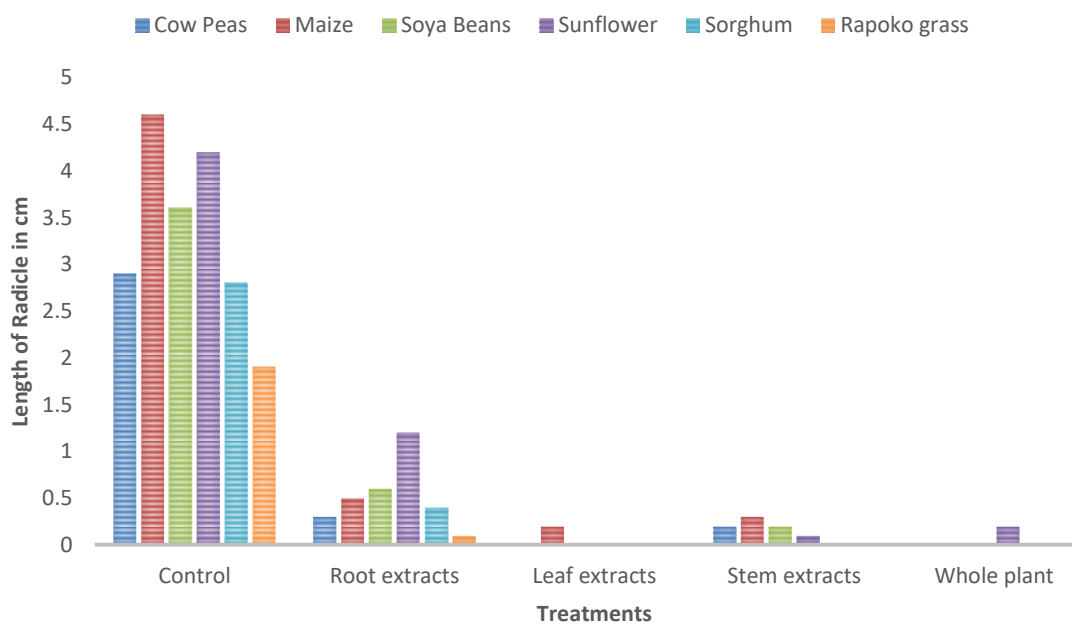
On the 10th day, there was a significant difference ( $p < 0.05$ ) in the length of the radicle among all treatments. The control treatment exhibited the greatest radicle growth on day 10. Slight radicle growth was observed in sunflower treated with root extracts. However, growth was completely inhibited in all crops treated with leaf, stem, and whole plant extracts by day 10. *T. diversifolia* parts showed a significant inhibitory effect on all crops on day 10 (Figure 3). The effects of *T. diversifolia* aqueous

extracts on radicle length on day 11 in the laboratory assay are illustrated in Figure 4.

On the 11th day, there was a significant difference ( $p < 0.05$ ) among all treatments in terms of the length of the radicle. However, there was no significant difference ( $p > 0.05$ ) observed among *T. diversifolia* aqueous extracts. While root extracts inhibited radicle growth, sunflower plants showed slight growth compared to all other crops. The greatest inhibition was observed with leaf and whole plant extracts, where no radicle growth was evident.



**Figure 3.** Length of the radicle on day 10 after treatment with the *T. diversifolia* extracts.



**Figure 4.** Length of the radicle on day 11 after treatment with the *T. diversifolia* extracts.

Radicle growth was observed in the control, but no radicle growth occurred in any of the other treatments (Figure 4). The effects of aqueous extracts on seed germination in the greenhouse assay are presented in Table 3.

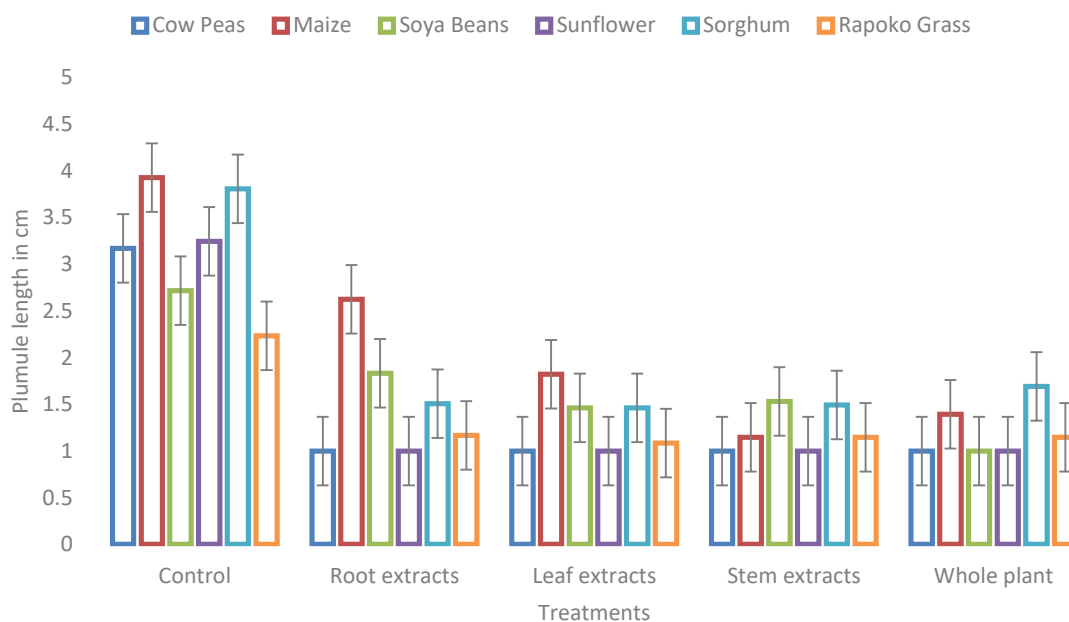
Aqueous extracts of *T. diversifolia* significantly reduced crop seed germination in the greenhouse. There was a significant difference ( $p < 0.05$ ) in the germination of crop seeds following the application of *T. diversifolia* parts in the greenhouse. The control exhibited the highest germination percentage among all treatments. The greatest inhibition was observed with leaf and stem

extracts, which substantially reduced germination across all test crops. Cowpeas were particularly inhibited in all extracts, and soybeans were notably affected by leaf and whole plant extracts. Root extracts also affected germination, with the lowest observed in rapoko and soybeans, although sunflower germination was not completely inhibited by root extracts. There was no significant difference ( $p < 0.05$ ) in the germination of all other crops except maize after treatment with root extracts (Table 2). Relatively low germination rates were observed in all crops treated with whole plant extracts, except for sunflower which showed more moderate

**Table 3.** Effects of *T. diversifolia* aqueous extracts on seeds germination in the greenhouse assay.

Treatment	Cow peas	Maize	Soya beans	Sunflower	Sorghum	Rapoko
Control	83.80 <sup>a</sup>	95.50 <sup>a</sup>	99.10 <sup>a</sup>	76.00 <sup>a</sup>	97.00 <sup>a</sup>	58.40 <sup>a</sup>
Root	10.00 <sup>b</sup>	46.50 <sup>b</sup>	19.10 <sup>b</sup>	10.00 <sup>b</sup>	18.50 <sup>b</sup>	19.10 <sup>b</sup>
Stem	10.00 <sup>b</sup>	13.50 <sup>c</sup>	19.10 <sup>b</sup>	10.00 <sup>c</sup>	27.60 <sup>b</sup>	13.50 <sup>c</sup>
Leaf	10.00 <sup>b</sup>	24.30 <sup>c</sup>	13.50 <sup>b</sup>	10.00 <sup>c</sup>	13.50 <sup>b</sup>	13.50 <sup>c</sup>
Whole plant	10.00 <sup>b</sup>	17.10 <sup>c</sup>	10.00 <sup>b</sup>	27.40 <sup>c</sup>	17.10 <sup>b</sup>	13.50 <sup>c</sup>
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by different letters are significantly different at 0.01 probability level.

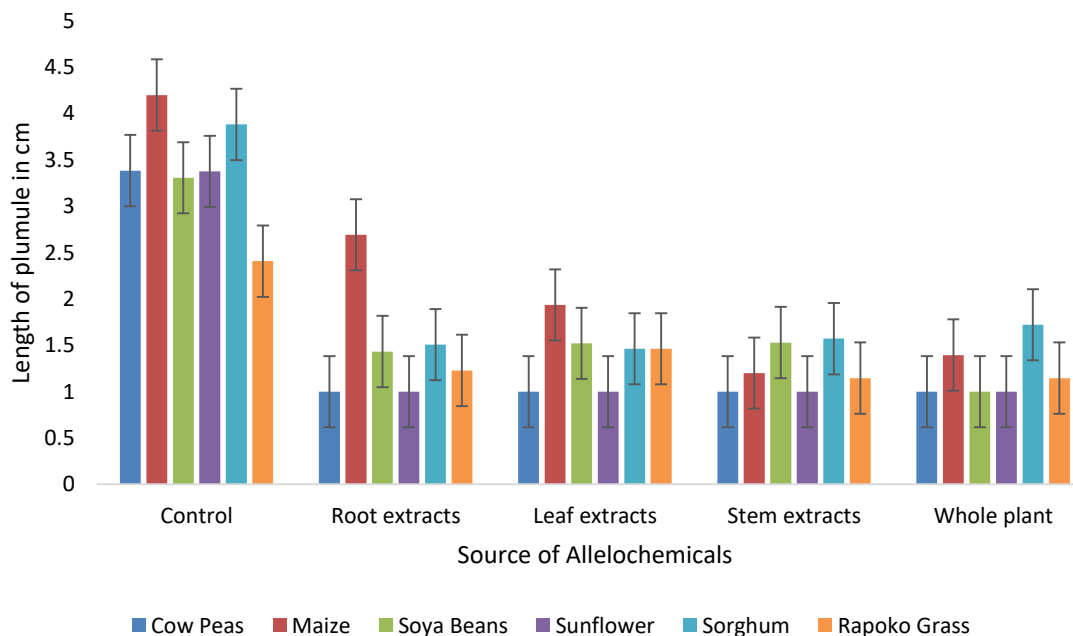
**Figure 5.** Length of the plumule on day 14 after treatment with extracts of *T. diversifolia*.

effects. This study confirms that aqueous extracts of *T. diversifolia* affect germination of crop seeds in the greenhouse. The effects of *T. diversifolia* aqueous extracts on plumule length on day 14 in the greenhouse assay are depicted in Figure 5.

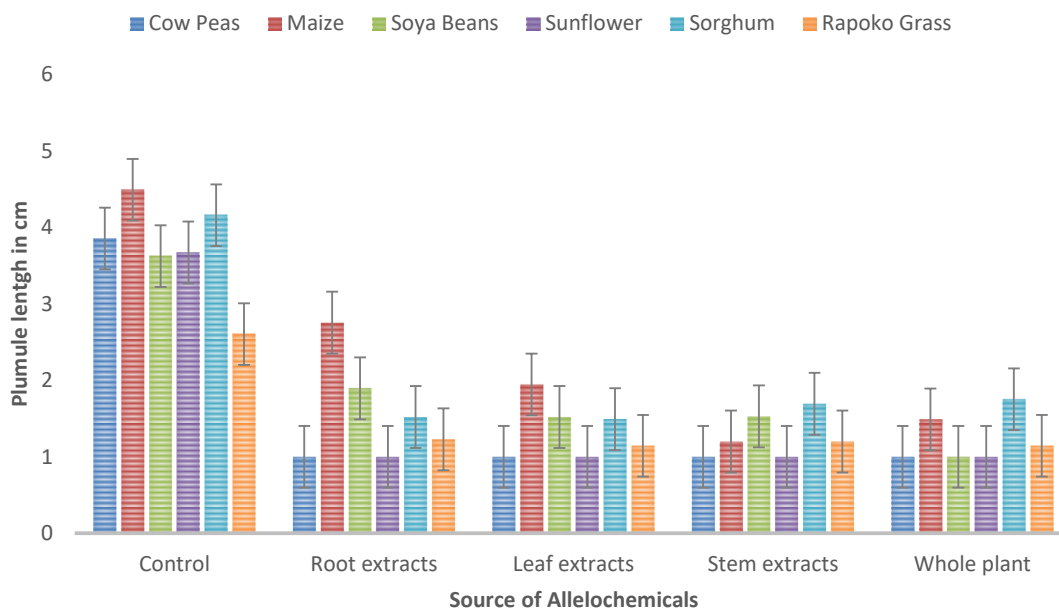
Results indicate that plumule growth of all crop seeds can be negatively affected by aqueous extracts of *T. diversifolia* on day 14 after planting. On the 14th day, there was a significant difference ( $p < 0.05$ ) in the length of the plumule among all treatments. The control exhibited the greatest plumule growth on day 14, while there was slight plumule growth observed in all treatments on that day. Maize showed the greatest growth with root extract on day 14, while sorghum exhibited the highest growth rate with whole plant extracts on the same day. There was no significant difference ( $p > 0.05$ ) in the length of the plumule of rapoko grass among all parts of *T. diversifolia* on day 14. Similarly, there was no significant difference ( $p > 0.05$ )

observed between cowpeas and sunflower in plumule growth across all *T. diversifolia* aqueous extract treatments. However, cowpeas and sunflower plumule growth were notably affected by the aqueous extracts of *T. diversifolia*. Overall, there was no significant difference ( $p > 0.05$ ) in the plumule growth of rapoko grass among all *T. diversifolia* aqueous extract treatments (Figure 5). Effects of *T. diversifolia* aqueous extracts on plumule length on day 15 in the Greenhouse assay are shown in Figure 6.

*T. diversifolia* extracts inhibited plumule growth on the 15th day after planting. There was a significant difference ( $p < 0.05$ ) between the control and all other treatments. The control exhibited the greatest plumule growth on day 15. Maize plumule growth was not completely inhibited by root and stem extracts. There was no significant difference ( $p > 0.05$ ) observed between the growth of cowpeas and sunflower across all *T. diversifolia* aqueous extracts. Similarly, there was no significant difference ( $p >$



**Figure 6.** Length of the plumule on day 15 after treatment with extracts of *T. diversifolia*.



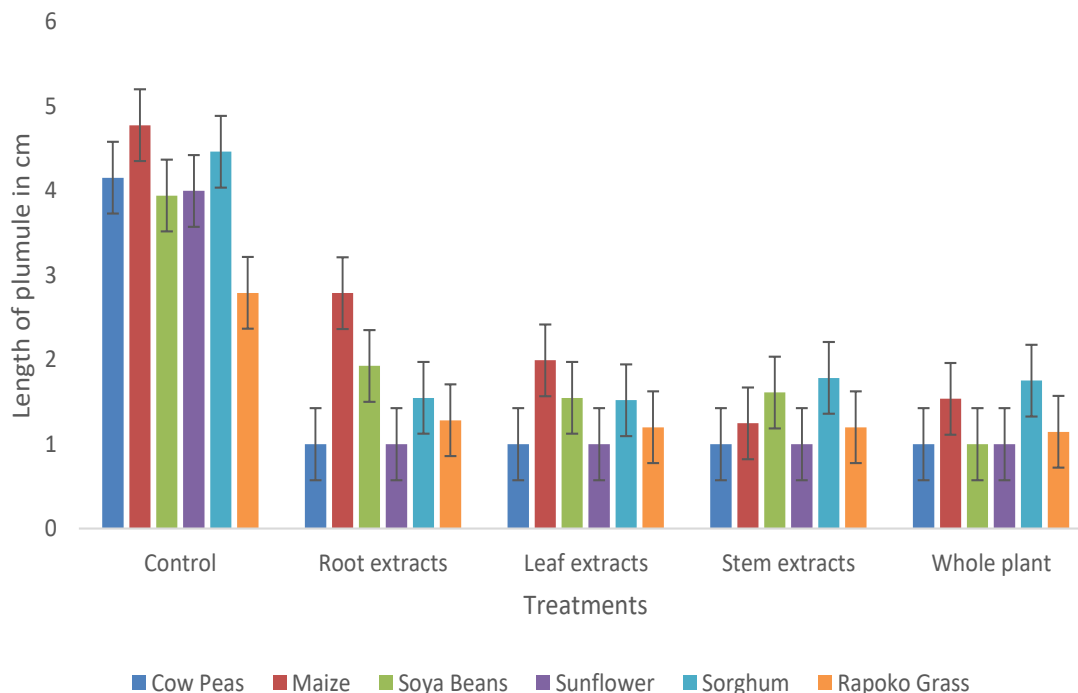
**Figure 7.** Length of the plumule on day 16 after treatment with extracts of *T. diversifolia*.

0.05) between soybeans and sorghum in root, leaf, and stem extracts. However, a significant difference was observed with whole plant extracts, where sorghum plumule growth was greater than soybean plumule growth. The plumule growth of rapoko grass was particularly affected by all *T. diversifolia* aqueous extracts except in leaf extracts, where it showed a slightly larger growth rate (Figure 6). The effects of *T. diversifolia*

aqueous extracts on plumule length on day 16 in the greenhouse assay are depicted in Figure 7.

On the 16th day, there was a significant difference ( $p < 0.05$ ) observed between the control and all other treatments. The greatest growth rate was observed in the control group. There was also a significant difference ( $p < 0.05$ ) among all the *T. diversifolia* treatments in terms of maize growth, which was particularly affected by stem





**Figure 8.** Length of the plumule on day 17 after treatment with extracts of *T. diversifolia*.

extracts. For cowpeas, there was no significant difference ( $p > 0.05$ ) between stem and whole plant extracts in terms of plumule growth, with the lowest plumule length observed in root extracts. Similarly, there was no significant difference ( $p > 0.05$ ) in plumule growth of soybeans between leaf and stem extracts, with the lowest growth seen in whole plant extracts.

Sunflower growth was significantly affected by *T. diversifolia* aqueous extracts on the sixteenth day, showing the lowest growth rate. However, there was no significant difference ( $p > 0.05$ ) among all the aqueous extracts of *T. diversifolia* on the length of the plumule of sorghum. Additionally, there was no significant difference ( $p > 0.05$ ) in the growth of rapoko grass among all *T. diversifolia* aqueous extracts (Figure 7).

The effects of *T. diversifolia* aqueous extracts on plumule length on day 17 in the greenhouse assay are shown in Figure 8.

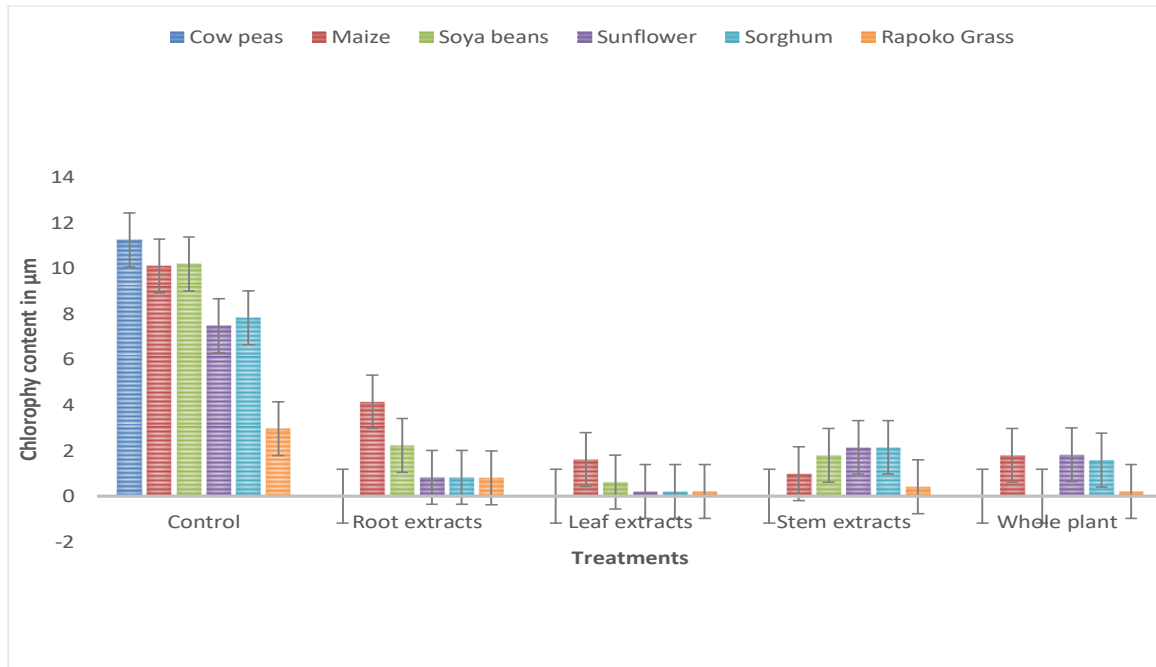
On the 17th day after planting, there was a significant difference ( $p < 0.05$ ) observed between the control and all other treatments in terms of plumule length. The control exhibited the greatest plumule growth among all treatments. Maize was less affected by root extracts compared to other crops on day 17. The plumule growth of sunflower and cowpeas was completely inhibited by extracts from *T. diversifolia*. However, there was no significant difference ( $p > 0.05$ ) in the growth of sorghum among all the parts of *T. diversifolia* on the 17th day after planting. Similarly, there was no significant difference ( $p > 0.05$ ) among *T. diversifolia* parts extracts in the growth of

rapoko grass on the 17th day after planting (Figure 8).

### Effects of aqueous extracts on chlorophyll of crop species in the greenhouse assay

Effects of *T. diversifolia* aqueous extracts on Chlorophyll content on day 30 in the Greenhouse assay are shown in Figure 9. Analysis of variance indicates that aqueous extracts of *T. diversifolia* have the potential to inhibit chlorophyll synthesis in all tested crop seeds on day 30. On the 30th day after planting, there was a significant difference ( $p < 0.05$ ) observed between the control and all other treatments in the chlorophyll content of cowpeas, maize, soybeans, sunflower, sorghum, and rapoko grass. The greatest inhibitory effects were observed with leaf extracts. Cowpeas were severely affected by the aqueous extracts of *T. diversifolia*, with two plants—one treated with leaf extracts and the other with composite extracts—reaching the permanent wilting point.

There was no significant difference ( $p > 0.05$ ) among sunflower, sorghum, and rapoko grass in terms of chlorophyll content in root and leaf extracts. Reduction in chlorophyll content was observed in rapoko grass across all aqueous extracts of *T. diversifolia*. Soybeans, sunflower, and sorghum exhibited only slight chlorophyll content in stem extracts. Soybean chlorophyll content was completely affected by whole plant extracts on the 30th day after planting. There was no significant difference ( $p > 0.05$ ) in the chlorophyll content among



**Figure 9.** Chlorophyll content of crop species in the greenhouse on day 30.

maize, sunflower, and sorghum in whole plant extracts (Figure 9).

### Effects of aqueous extracts on crop species in the greenhouse assay

Effects of *T. diversifolia* aqueous extracts on Stem girth at day 30 after planting in the Greenhouse assay are shown in Figure 10. On day 30, there was a significant difference ( $p < 0.05$ ) observed between the control and all other treatments in terms of stem girth. The control exhibited the greatest stem girth. However, there was no significant difference ( $p > 0.05$ ) in the stem girth of rapoko grass among all treatments.

Similarly, there was no significant difference ( $p > 0.05$ ) among cowpeas, soybeans, sunflower, and rapoko grass in terms of stem girth across all *T. diversifolia* extracts. Cereal crops showed a slight expansion of stem girth in all aqueous extracts. Among the *T. diversifolia* treatments, root extracts exhibited a slightly inhibitory effect on the stem girth of maize. Stem extracts also showed a slight inhibitory effect on the stem girth of sorghum on day 30 after planting. These results clearly indicate that *T. diversifolia* has a negative impact on stem girth on day 30 after planting (Figure 10). Figure 11 presents the results of the aqueous extracts on leaf biomass in the greenhouse plants.

On day 30 after planting, there was a significant difference ( $p < 0.05$ ) observed between the control and all other treatments in terms of leaf biomass. Maize leaf

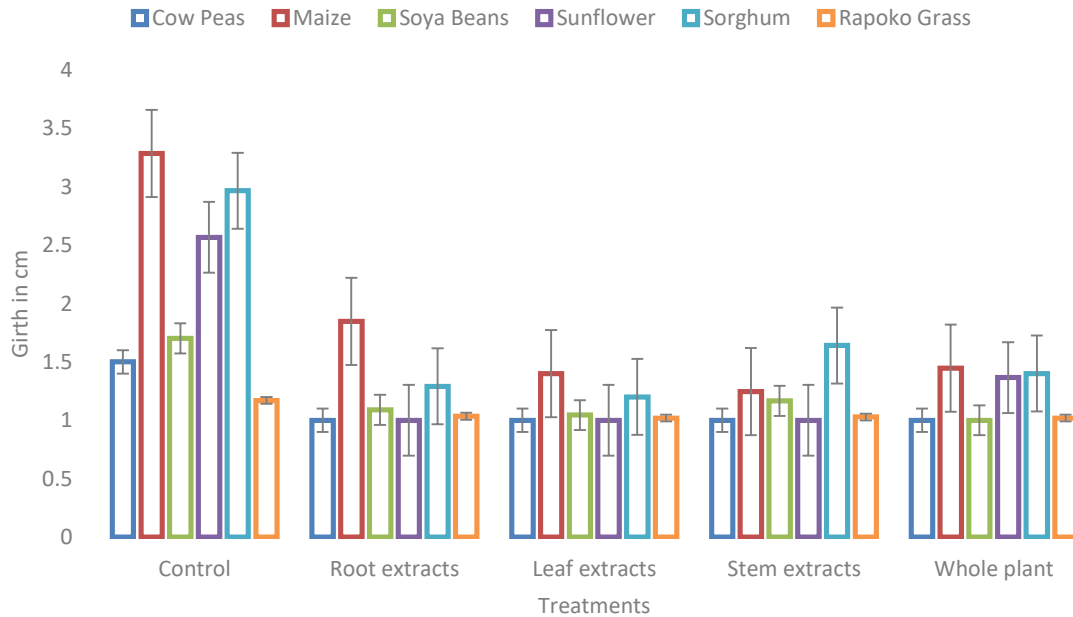
biomass was not completely inhibited by root extracts, but complete inhibition was observed with leaf, stem, and whole plant extracts. Leaf biomass of rapoko grass was entirely inhibited by *T. diversifolia* extracts. These results demonstrate that leaf biomass of all test crops was affected by aqueous extracts of *T. diversifolia* on day 30 after planting, which negatively impacts the production of photosynthetic material and compromises overall productivity (Figure 11). Figure 12 presents the effects of *T. diversifolia* extracts on root biomass at 30 days after planting.

On day 30, there was a significant difference ( $p < 0.05$ ) observed between the control and all other treatments in terms of the root biomass of the test crops. The control exhibited the greatest root biomass. The most inhibitory effects were observed with leaf extracts.

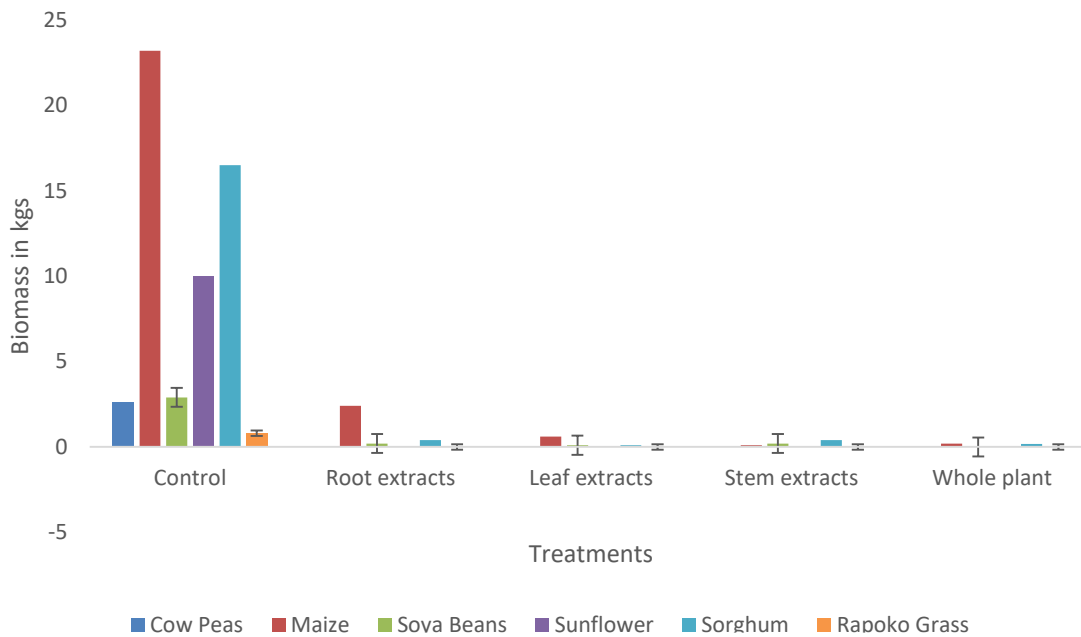
There was no significant difference ( $p > 0.05$ ) among all crop treatments in terms of root biomass affected by leaf extracts. Maize showed greater root biomass in root and whole plant extracts. Additionally, there was no significant difference ( $p > 0.05$ ) observed among cowpeas, soybeans, sunflower, sorghum, and rapoko grass in response to all the aqueous extracts of *T. diversifolia* (Figure 12).

## DISCUSSION

*T. diversifolia* leaves and whole plant extracts exhibited the greatest inhibition on the germination of maize, sunflower, soybeans, sorghum, rapoko grass, and



**Figure 10.** Stem girth at day 30 after planting in the greenhouse.

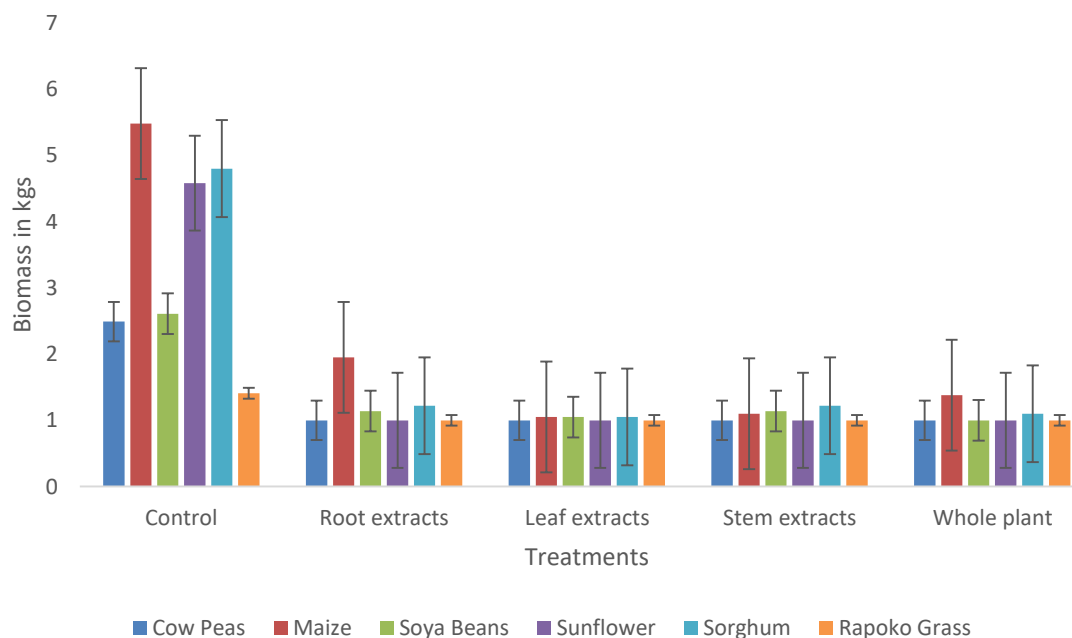


**Figure 11.** Leaf biomass on the 30th day after planting.

cowpeas in both greenhouse and laboratory conditions. In the laboratory, leaves and stems were the most potent suppressors of crop seed germination (Figure 7). In the greenhouse, sorghum germination was less affected by whole plant extracts compared to other crops, although root extracts significantly inhibited germination across various crops. Similar findings were reported by Shackleton et al. (2019), who identified ferulic acid,

syringic acid, and chlorogenic chemicals in *T. diversifolia* responsible for affecting morphological processes and germination in many plants.

According to Musyimi et al. (2012), allelochemicals present in *T. diversifolia* inhibit water absorption critical for germination by hydrolyzing food constituents within seeds. Additionally, the allelochemical complex within *T. diversifolia* may include compounds that inhibit peroxidase,



**Figure 12.** Root biomass on the 30th day after planting.

alpha-amylase, and acid phosphatases, enzymes crucial for starch breakdown during seed germination. Various concentrations of *T. diversifolia* parts demonstrated inhibitory effects on the germination and seedling growth of maize, soybeans, cowpeas, and sunflower, highlighting that allelopathic effects are dose-dependent and vary among plant species.

The study emphasized that soybeans, rapoko grass, and cowpeas were among the most inhibited plants, consistent with previous research indicating high susceptibility of cowpeas to allelochemicals (Shackleton et al., 2019). Allelopathic compounds are known to impact various physiological processes such as cell turgor, photosynthesis rate, enzyme activity, metabolic energy, mitosis, DNA replication, protein and hormone synthesis, mineral absorption, chlorophyll synthesis, membrane permeability of chloroplasts and mitochondria, abscisic acid levels, lipid peroxidation, and overall cell growth (Bano et al., 2012).

The study concluded that *T. diversifolia*'s impact on reducing germination ensures its dominance in habitats where it is the sole occupier, underscoring the broad inhibitory effects of its aqueous extracts on crop seed germination.

The stem girth of all test crops was completely inhibited by the aqueous extracts of *T. diversifolia*. Among all the test crops, rapoko grass was the most affected, suggesting an impending food scarcity in areas infested with *T. diversifolia*. This situation could have normative impacts on both the leather and meat industries.

The study clearly indicated that both root and leaf biomass is threatened by *T. diversifolia* infestation, as

evidenced by complete inhibition of biomass in all test crops. Leaf biomass was significantly more inhibited than root biomass across all aqueous extracts of *T. diversifolia*.

Given this scenario, it implies that during decomposition, high concentrations of *T. diversifolia* will be released into the environment, impeding the germination and growth of plants in proximity. Against this backdrop, the weed must be completely eliminated from cropping fields; if left unchecked, it has the capacity to colonize all native species and become dominant.

Moreover, the weed exhibits strong attributes of an invasive species that alters the environment with adverse effects of its allelochemicals, eventually leading to its sole occupancy of invaded lands. The inhibitory effects of *T. diversifolia* were evaluated by examining the average inhibition caused by *T. diversifolia* leaves and whole plant extracts, which showed the greatest inhibition on the germination of maize, sunflower, soya beans, sorghum, rapoko grass, and cowpeas in both the greenhouse and laboratory settings. In the laboratory, the leaves and stems were the most potent suppressors of crop seed germination (Figure 7). In the greenhouse, sorghum germination was not highly affected by whole plant extracts, although other crops were significantly affected by root extracts.

Similar findings were reported by Shackleton et al. (2019), who confirmed that *T. diversifolia* contains ferulic acid, syringic acid, and chlorogenic chemicals that affect various morphological processes in plants, including germination.

According to Musyimi et al. (2012), allelochemicals in *T. diversifolia* inhibit water absorption by hydrolyzing food

constituents within seeds. Additionally, the allelochemical complex within *T. diversifolia* extracts may include compounds that inhibit enzymes like peroxidase, alpha-amylase and acid phosphatases, which aid in starch breakdown during seed germination.

All extracts from *T. diversifolia* parts suppressed radicle growth in maize, sunflower, soya beans, sorghum, rapoko grass, and cowpeas, with the greatest inhibition observed in whole plant and leaf extracts (Figure 1). Radicle length of germinated seedlings was reduced by these extracts, confirming the presence of mitotic inhibitors within the allelochemical complex of *T. diversifolia*. Due to its ability to inhibit germination, root and shoot growth of affected crops, *T. diversifolia* ends up dominating plantations.

Maize is a staple crop in Zimbabwe's semi-arid regions, and these results imply that allelopathy is a functional trait in *T. diversifolia* that contributes to altering habitats, favoring its dominance. Thus, allelopathy serves as one of the strongest mechanisms in *T. diversifolia*'s arsenal for dominating agroecosystems (Figure 2).

## Conclusions

The study demonstrated the allelopathic effects of leaf, stem, whole plant, and root extracts of *T. diversifolia* on the germination and seedling growth of four selected crops. Consequently, *T. diversifolia* leaf, stem, whole plant, and root extracts possess herbicidal properties that significantly affected the germination and growth of these crops. The aqueous leaf and whole plant extracts exhibited greater allelopathic effects compared to the aqueous stem and root extracts. All four selected crops exhibited growth stunting after treatment with the aqueous leaf and whole plant extracts. *T. diversifolia* has proven its potential for use in weed management programs. Further studies to evaluate other allelopathic compounds produced by *T. diversifolia* may therefore be necessary.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors would like to thank the Kushinga Phikelela Poly Technic for providing sites for the experiments.

## REFERENCES

- Bano S, Ullah MA, Khaliq A, Abbasi KH, Khanum S (2012). Effects of aqueous extract of sun-dried neem (*azadirachta indica*) leaves on wheat and wheat weeds (wild oat and dumbi sitti) in Vitro. International Research Journal of Pant Science 3(4):69-73.
- Carboni M, Münkemüller T, Lavergne S, Choler P, Borgy B, Violle C, Essl, F, Roquet, C, Munoz F, DivGrass C, Thuiller W (2016). What it takes to invade grassland ecosystems: Traits, introduction history and filtering processes. Ecology letters 19(3): 219-229.
- Chukwuka KS, Obiakara MC, Ogunsumi IA (2014). Effects of aqueous plant extracts and inorganic fertilizer on the germination, growth and development of maize (*Zea mays* L.). Journal of Agricultural Sciences, Belgrade 59(3): 243-254.
- Gharde Y, Singh PK, Dubey RP, Gupta, PK (2018). Assessment of yield and economic losses in agriculture due to weeds in India. Crop Protection 107:12-18.
- Muoghalu JI, Chuba DK (2005). Seed germination and reproductive strategies of *Tithonia diversifolia* (Hemsl.) Gray and *Tithonia rotundifolia* (PM) Blake. Applied Ecology and Environmental research 3(1):39-46.
- Musyimi DM, Kahihu SW, Buyela DK, Sikuku PA (2012). Allelopathic effects of Mexican sunflower [*Tithonia diversifolia* (Hemsl.) A. Gray] on germination and growth of Spiderplant.
- Olakojo SA (2004). Evaluation of some maize inbred lines for tolerance to *Striga lutea* (Lour) in Southern Guinea savanna ecology. Journal of Food Agriculture and Environment 2:256-259.
- Otusanya O, Ilori O (2014). Studies on the allelopathic effects of *Tithonia rotundifolia* on the germination and seedling growth of some legumes and cereals. International Journal of Biology 6(1):38-47.
- Perkins LB, Nowak RS (2013). Invasion syndromes: hypotheses on relationships among invasive species attributes and characteristics of invaded sites. Journal of Arid Land 5:275-283.
- Pyšek P, Richardson DM (2007). Traits associated with invasiveness in alien plants: where do we stand? Biological Invasions pp. 97-125.
- Rejmánek M, Huntley BJ, Le Roux JJ, Richardson DM (2017). A rapid survey of the invasive plant species in western Angola. African Journal of Ecology 55(1):56-69.
- Rejmánek M, Richardson DM (2013). Trees and shrubs as invasive alien species—2013 update of the global database. Diversity and distributions 19(8):1093-1094.
- Rodenburg J, Demont M, Zwart SJ, Bastiaans L (2016). Parasitic weed incidence and related economic losses in rice in Africa. Agriculture, Ecosystems & Environment 235:306-317.
- Ronald M, Peter J, Charles K, Tibugari H (2011). Integrated weed management in Zimbabwe's smallholder sector, where are we? a review. Modern Applied Science 5(5):111.
- Rugare JT (2018). Allelopathic effects of green manure cover crops on the germination and growth of blackjack (*Bidens pilosa* L.) and rapoko grass [*Eleusine indica* (L.) Gaertn] (Doctoral dissertation, Stellenbosch: Stellenbosch University).
- Shackleton RT, Nunda W, Beale T, Van Wilgen BW, Witt AB (2019). Distribution of invasive alien *Tithonia* (Asteraceae) species in eastern and southern Africa and the socioecological impacts of *T. diversifolia* in Zambia. Bothalia-African Biodiversity & Conservation 49(1):1-11.
- Valkó O, Deák, B, Török P, Kelemen A, Miglécz T, Tóth K, Tóthmérés B (2016). Abandonment of croplands: problem or chance for grassland restoration? Case studies from Hungary. Ecosystem Health and Sustainability 2(2):e01208.
- Yaduraju NT, Rao AN (2013). Implications of weeds and weed management on food security and safety in the Asia-Pacific region. pp. 13-30.