

Allelopathic Effects of *Tithonia diversifolia* Extracts on Biochemical Parameters and Growth of *Vigna unguiculata*

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Abstract

Allelopathy has gained much attention in recent times in the sustainable agricultural systems. Allelochemicals released by different plants interact differently with each other, it therefore becomes imperative to study the possible biochemical processes underlying the allelopathy of some plants on other plants. In the present study, investigation was carried out on the phytochemical composition of aqueous and methanolic extracts of *Tithonia diversifolia* and their allelopathic effect on the growth of *Vigna unguiculata*. Phytochemical analyses indicated the presence of cardiac glycosides, flavonoids, and alkaloids in the methanolic extract, and in addition anthraquinones and saponins in the aqueous extract. The methanolic extract was found to be more phytotoxic than the aqueous extract; and the seed germination and seedling growth inhibition was concentration dependent. Both the methanolic and aqueous extracts increased significantly the concentration of proline and the level of antioxidant enzymes (superoxide dismutase and catalase) in *Vigna unguiculata*, while protein concentration and chlorophyll content were significantly reduced. There was however no significant difference in the amount of total soluble sugar. Cytological examination of the root tips of *Allium cepa* treated with both extracts showed no disparity in mitotic index and there was no chromosomal aberration. The study concluded that the extracts of *T. diversifolia* affected the seed germination and growth of *Vigna unguiculata*.

Keywords: Allelochemicals, Allelopathy, aqueous extracts, biochemical responses, methanolic extracts, phytochemicals, *Tithonia diversifolia*, *Vigna unguiculata*

1. Introduction

Allelopathy is a phenomenon whereby secondary metabolites synthesized by fungi, viruses, microorganisms and plants influence biological and agricultural systems, which may be either stimulatory or inhibitory (Farooq et al., 2011). Allelochemicals are products of the secondary metabolism and are non-nutritional primary metabolites (Iqbal & Fry, 2012). These compounds belong to numerous chemical groups including triketones, terpenes, benzoquinones, coumarins, flavonoids, terpenoids, strigolactones, phenolic acids, tannins, lignin, fatty acids and non-protein amino acids. A wide range of these biochemicals are synthesized during the shikimic pathway, or in the case of essential oils, from the sopenoid pathway (Hussain & Reigosa, 2012). Allelochemicals are known to be released from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes (Kruse et al., 2000). The detrimental effects of allelochemicals on plant germination and growth have been reported by many investigators, among whom are Onwugbuta-Enyi (2001), Ismail and Chong (2002), Bais et al. (2003), Zahida et al. (2006), Bogatek et al. (2006) and Ilori et al. (2007). The detrimental effects reported by these investigators are secondary expressions of primary effects on metabolic processes such as photosynthesis, respiration, cell division, pigment synthesis, production of plant hormones and their balance, membrane stability and permeability, mineral uptake, movement of stomata, amino acid synthesis, nitrogen fixation, and specific enzyme activities (Baerson et al., 2005).

Researches carried out on the allelopathic effect of *Tithonia diversifolia* on crops showed that *Tithonia diversifolia* could either have beneficial or detrimental effect on plant's growth. However, emphasis has been on the physical parameters of growth such as shoot length, leaf area, fresh shoot weight and dry shoot weight (Otusanya & Ilori, 2012). Cell growth in plants depends on normal mitotic processes. Following DNA synthesis, mitosis and cytokinesis occur; mitotic activity, alterations in the mitotic phase and chromosomal aberrations are key

parameters by which plant growth may be evaluated. Cytogenetic assays for evaluation of these parameters are commonly carried out using *Allium cepa* (onion) test. Due to the action of allelochemicals contained in allelopathic plants, a large number of physiological functions and biochemical reactions in plants are affected such as seed germination, cell division, cell elongation, membrane permeability and ion uptake (Tomita-Yokotani et al., 2005; Setia et al., 2007). Consequently, the effects of allelochemicals can be detected at molecular, structural, biochemical, physiological and ecological levels of plant organization (Gniazdowska & Bogatek, 2005).

This study is therefore designed to investigate some of the allelopathic effects of *Tithonia diversifolia* on biochemical parameters and growth of *Vigna unguiculata* which may underlie its visible physiological effect.

2. Materials and Methods

2.1 Collection of Plant Material

The fresh shoots of *Tithonia diversifolia* were collected along Ede road, Ile-Ife between July and September, 2013; while the seeds of *Vigna unguiculata* were purchased from a local market in Ile-Ife, Nigeria. The plants were identified at the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife with specimen voucher No: 17531.

2.2 Preparation of Extracts of *T. diversifolia*

Fresh shoot of *T. diversifolia* were mechanically ground into paste. The paste was suspended in 80% methanol (1:10 w/v) for 72 hr with occasional agitation. The suspension was filtered using sieve cloth and concentrated to dryness 'in vacuo' at 40°C in a rotary evaporator to obtain the methanolic extract.

Fresh shoot of *T. diversifolia* were cut into small chips and finely ground with mortar and pestle. The ground plant material was soaked in 10 volumes of distilled water for 48 hr with occasional agitation. The solution was filtered through cheese cloth to remove debris and finally filtered using Whatman No.1 filter paper to obtain the aqueous extract. This filtrate was prepared daily and kept in the refrigerator to prevent the degradation of the allelochemicals.

The methanolic and aqueous extracts were phytochemically screened for the presence of secondary metabolites using standard procedures based on the methods described by Sofowora (1993) and Trease and Evans (2002).

2.3 Germination Experiment

The effects of the extracts of *T. diversifolia* on seed germination of *Vigna unguiculata* was carried out following the method of Li and Yang (2006). The seeds of *V. unguiculata* were decontaminated by soaking for 10 min in 5% sodium hypochlorite, rinsed for 5 min in running water and finally washed in distilled water. Five uniform seeds were randomly selected and placed in each of 18 clean Petri dishes which have been lined with cotton wool and sterilized Whatman No. 1 filter paper. The cotton wool served as an absorbent for water or the extracts used as treatments so as to keep the seeds moistened always and at the same time avoid the imposition of water stress. Six Petri dishes were moistened with 10 ml distilled water and served as the control, another six were moistened with 10 ml of the methanol extract (20, 40, 60 and 80 mg ml⁻¹) and the remaining six Petri dishes were moistened with 10 ml of 25, 50 and 100% of the aqueous extract. The Petri dishes were incubated at 27°C with good lightning for 96 hr. Emergence of 1 mm radicle was used as the criterion for germination. Daily measurements of the plumule, root and shoot lengths of germinated seeds were taken. Germination % in treatment groups was calculated thus:

$$\text{Germination (\%)} = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

2.4 Decomposition Study

Decomposition study of the leaves of *T. diversifolia* was carried out as method described by Teerarak et al. (2010). Loam soil was collected from an agricultural field. Oven-dried plant leaves were ground into small pieces and mixed into the soil in polythene bags. Three concentrations of the leaves (2.5, 5, 10 g dry weight per 100 g soil) were mixed with soil in the pots and allowed to decompose naturally. Negative control pots were filled with the same soil but without leaf debris. Distilled water (30 ml) was added to each pot to keep the soil moist throughout the decomposition periods of 0 (when seeds were sown immediately after leaf debris was mixed into soil), 1, 2, 3, and 4 weeks. Five viable seeds of *V. unguiculata* were sown separately in the pots and watered regularly. Seedling emergence was recorded 7 days after planting, and experimental data was converted into a percentage of the control. A complete randomized design with three replicates was used for the decomposition studies.

2.5 Growth Experiment and Biochemical Analysis

The study of biochemical response of *V. unguiculata* to aqueous and methanolic extracts of *T. diversifolia* was carried out as follows:

Forty two plastic pots were prepared by perforating the base of the pots and filled with loam and sand soil, the perforation was to prevent the pots from water logging. Fifteen viable seeds of *V. unguiculata* were sown in each of the pots and were kept such that full sunlight for eight hours was ensured and watering was done morning and evening. After 7 days of planting, *V. unguiculata* was harvested and analyzed. The pots were then randomly selected into 7 treatment groups with 6 pots in each group. Groups I – III were treated daily with appropriate volume of varying concentrations (20, 40 and 80 mg ml⁻¹) of the methanolic extract, groups IV – VI, with 25, 50 and 100% of the aqueous extract of *T. diversifolia*, and group VII with distilled water and served as the control. The plants were harvested for analysis every five days for thirty days, plant shoot height, leaf length and leaf breadth were also measured. For every harvest, the plant sample was analyzed for protein, carbohydrate and chlorophyll contents, proline concentration, catalase and superoxide dismutase activities.

2.5.1 Chlorophyll Concentration

Determination of the chlorophyll content followed the method of Witham et al. (1971) as described by Opabode and Akinyemiju (2007). Chlorophyll was extracted from 1 g of the leaf tissue using 20 ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 5 min. The extraction was repeated until the residue became colorless. The absorbance of the extract was taken at 647 and 664nm against 80 % acetone blank. The amount of chlorophyll 'a', chlorophyll 'b' and total chlorophyll was calculated as follows:

$$\text{Chlorophyll a } (\mu\text{M g}^{-1} \text{ dry weight}) = 13.19A_{664} - 2.57A_{647} * V/1000 * W$$

$$\text{Chlorophyll b } (\mu\text{M g}^{-1} \text{ dry weight}) = 22.10A_{647} - 5.26A_{664} * V/1000 * W$$

$$\text{Total Chlorophyll } (\mu\text{M g}^{-1} \text{ dry weight}) = 7.93A_{664} - 19.53A_{647} * V/1000 * W$$

Where:

V is final volume of chlorophyll solution;

W is weight of fresh leaves;

A₆₆₄ and A₆₄₇ are absorbance at 664nm and 647nm wavelength in a visible spectrophotometer.

2.5.2 Total Soluble Sugar

The total soluble sugar was determined using the method of Dubois et al. (1951) as described by Marmit and Sharma (2008). The plant shoot (500 mg) was homogenized with 10 ml of 80% ethanol and centrifuged at 2000 rpm for 20 min, the supernatant was collected. The standard curve was prepared with glucose (0.5 mg ml⁻¹). 2% phenol solution was added, mixed and then 96% sulphuric acid was added rapidly. The tube was gently agitated during the addition of the acid and then allowed to stand in a water bath at 26 - 30°C for 20 min. The absorbance was taken at 490nm in a spectrophotometer. Amount of soluble sugar was calculated from the standard plot.

2.5.3 Protein Content

The protein content was measured according to the method of Lowry et al. (1951) using bovine serum albumin (2 mg ml⁻¹) as standard.

2.5.4 Proline Concentration

Estimation of proline concentration in the plant shoot was done according to the method of Bates et al. (1973) as described by Celik and Atak (2012). The plant shoot (0.1 g) was homogenized with 5 ml 3% (w/v) sulphosalicylic acid and centrifuged (4°C) at 4000 rpm for 10 min. The reaction mixture [supernatant (2 ml), glacial acetic acid (2 ml), and ninhydrin reagent (2 ml) was mixed thoroughly and incubated for 1 hr in a water bath. The mixture was then cooled to room temperature; 4 ml of toluene was added, mixed properly and allowed to stand for 10 min. The brick-red toluene layer was removed and absorbance was read at 520nm against toluene blank.

2.5.5 Catalase

The activity of catalase in the plant shoot was determined following the procedure of Cakmak and Marschner (1992). Approximately 0.5 g plant shoot was homogenized in 8 ml of solution containing 50 mM PBS (pH 7.6) and 0.1 mM Na-EDTA and then centrifuged for 15 min at 20,000 g at 4°C. The reaction mixture (1.0 ml) was prepared by adding 0.1 ml of 50 mM PBS pH 7.6, 0.1 ml of 0.1 mM EDTA, 0.1 ml of 100 mM H₂O₂ and 0.7 ml of enzyme aliquot. The degradation rate of H₂O₂ was monitored for 3 min every 30 sec at 240nm.

2.5.6 Superoxide Dismutase

The level of SOD activity in the plant shoot was determined as follows: The homogenate (200 µl) was diluted in 800 µl of distilled water to make a 1:5 dilution. An aliquot of 200 µl of the diluted sample was added to 2.5 ml of 0.05 M carbonate buffer (pH 10.2) in the spectrophotometer and the reaction was initiated by the addition of 300 µl of freshly prepared 0.3 mM adrenaline to the mixture and was quickly mixed by inversion. The reference cuvette contained 2.5 ml buffer, 300 µl of substrate (adrenaline) and 200 µl of water. The increase in absorbance at 480nm was monitored every 30 sec for 3 min. (Misra & Fridovich, 1972)

The SOD activity was estimated as follows:

$$\text{Increase in absorbance per minute} = A_3 - A_0/2.5$$

Where:

A_0 = absorbance after 30 seconds, A_3 = absorbance after 150 seconds

% inhibition = $100 - 100 * (\text{increase in absorbance for substrate} / \text{increase in absorbance for blank})$

1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline.

2.6 Cytogenetic Assay

Healthy and equal-sized bulbs of *Allium cepa* L. (onions) were used. Outer scales were removed from the onions and basal ends were cut and the bulbs were placed in containers with their basal ends dipped in distilled water and germinated under standard laboratory conditions. The newly emerged roots were treated with graded concentrations of the methanolic (2.5, 5 and 10 mg ml⁻¹) and aqueous (25%, 50% and 100%) extracts of *T. diversifolia* for 24, 48 and 72 h. The negative control group was treated with distilled water and the positive control group treated with sodium benzoate (100 µg ml⁻¹). At the end of each exposure period, root tips were cut and fixed in a freshly prepared mixture of ethanol and acetic acid (3:1 v/v) for 24 h, hydrolysed for 10 min with 18% HCl and stained with FLP Orcein reagent at 37°C for 20 min and squashed on grease-free slide for cytological examination of the root tip cells using a binocular microscope (Olympus, USA). The mitotic index was calculated as the ratio between dividing cells and examined total cells. The frequency of each mitotic phase was calculated as the percentage in relation to divided cells counted in mitosis. The degree of chromosomal aberration was measured as the frequency of aberrant cells, defined as the ratio of number of aberrant cells to total number of cells examined.

2.7 Statistical Analysis

Data generated were subjected to an analysis of variance, and means were compared using the LSD test at a 5% level of significance.

3. Results

The phytochemical constituents of *T. diversifolia* showed the presence of saponin, flavonoids and anthraquinones in the aqueous extract while methanolic extract showed the presence of alkaloids, cardiac glycosides and flavonoids. Flavonoids were found to be present in both aqueous and methanolic extracts (Table 1).

The effect of decomposed leaf residue of *T. diversifolia* on the germination of *V. unguiculata* seed is as shown in Figure 1. The germination was affected by the amount of leaves decomposed in the soil. The germination of *V. unguiculata* significantly reduced ($p < 0.01$) with increasing concentration of both aqueous and methanolic extracts (Figure 2a and 2b). The methanolic extract had a greater inhibitory effect on the germination of *V. unguiculata* than the aqueous extract. The effect of the extracts of *T. diversifolia* on the germination of *V. unguiculata* showed a concentration dependent effect.

Table 1. Phytochemical Constituents of *Tithonia diversifolia* Extracts

Phytochemicals	Extracts	
	Methanolic	Aqueous
Alkaloids	+	-
Saponins	-	+
Cardiac Glycosides	+	-
Tannins	-	-
Flavonoids	+	+
Anthraquinones	-	+

Note: Present +, Absent -

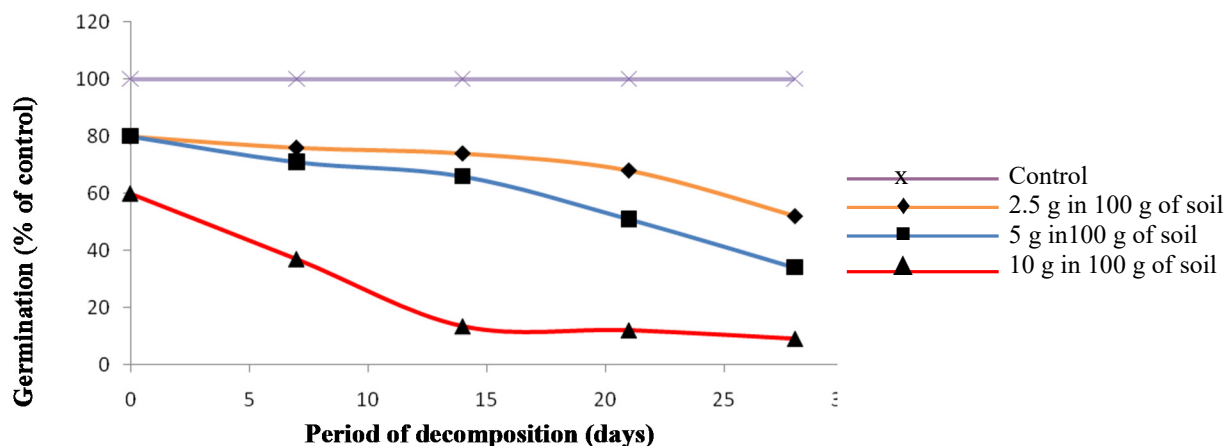


Figure 1. Effect of Decomposed Leaf Residue of *Tithonia diversifolia* on the Germination of *Vigna Unguiculata* seed

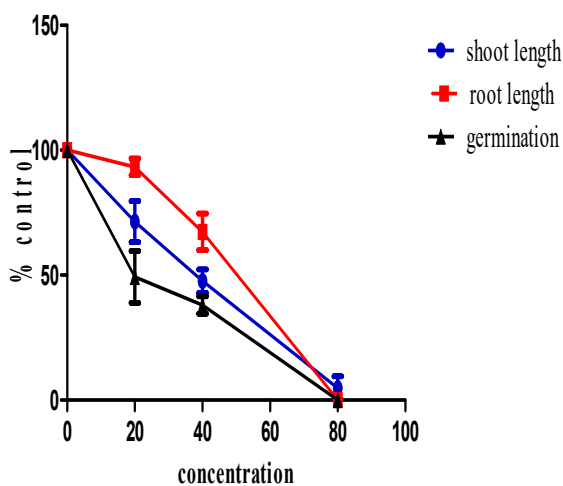


Figure 2a. Effect of Methanolic Extract of *Tithonia diversifolia* on the Germination of *Vigna unguiculata* seed

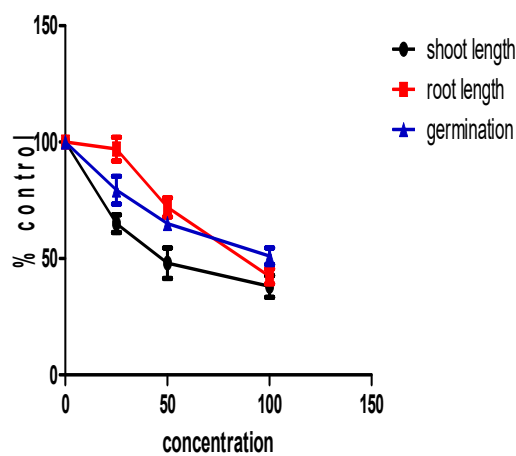


Figure 2b. Effect of Aqueous Extract of *Tithonia diversifolia* on the Germination of *Vigna unguiculata* seed

Treatment with both aqueous and methanolic extracts of *T. diversifolia* increased proline concentration significantly for the first five days by 69%, 67.4% and 77.2% for 25, 50 and 100% aqueous extracts respectively and by 25%, 63.3% and 63.1% for 20, 40 and 80 mg ml⁻¹ methanolic extract respectively, but marginally thereafter (Figure 3). Aqueous extract of *T. diversifolia* increased protein concentration by 47.7%, 65.4% and 54.5% for 25, 50 and 100% respectively; however increase from 5th to 30th day was insignificant. Treatment with methanolic extract of *T. diversifolia* increased protein concentration by 50%, 41.2% and 35.5% for 20, 40 and 80 mg ml⁻¹ respectively; it was however marginal for 40 and 80 mg ml⁻¹ from 10th to 30th day (Figure 4). The effect of aqueous extract of *T. diversifolia* on total chlorophyll is as shown in Figure 5. Methanolic extract decreased the amount of total chlorophyll while aqueous extract increased the amount of total chlorophyll. Treatment with aqueous extract of *T. diversifolia* increased the activity of catalase on the 10th day (18.9% and 9.1% for 50 and 100% concentration respectively, with marginal increase in the 25% concentration), thereafter the activity decreased. Methanolic extract increased the activity of catalase by 78.6%, 78.6% and 81.3% for 20, 40 and 80 mg ml⁻¹ concentration respectively on the 10th day; however, there was marginal increase thereafter (Figure 6). The superoxide dismutase activity increased by about 0.3 units mg⁻¹ of protein for 25%, 50% and 100% concentration of aqueous extract after 5 days of treatment; and by about 0.5 units mg⁻¹ of protein from the 10th day to the 30th day of treatment. In the group treated with methanolic extract, there was an increase on the 15th day by about 1.23, 1.7, 1.7 units mg⁻¹ of protein for 20, 40 and 80 mg ml⁻¹ respectively; however, there was marginal increase thereafter (Figure 7). Treatment with extracts of *T. diversifolia* had no significant effect on the total soluble sugar (Figure 8).

Cytogenetic studies using *Allium cepa* test showed that there was no significant difference in the mitotic index values of the control and the onion roots treated with both aqueous and methanolic extracts of *T. diversifolia*. The cells of both the treated and control groups also showed no chromosomal aberrations.

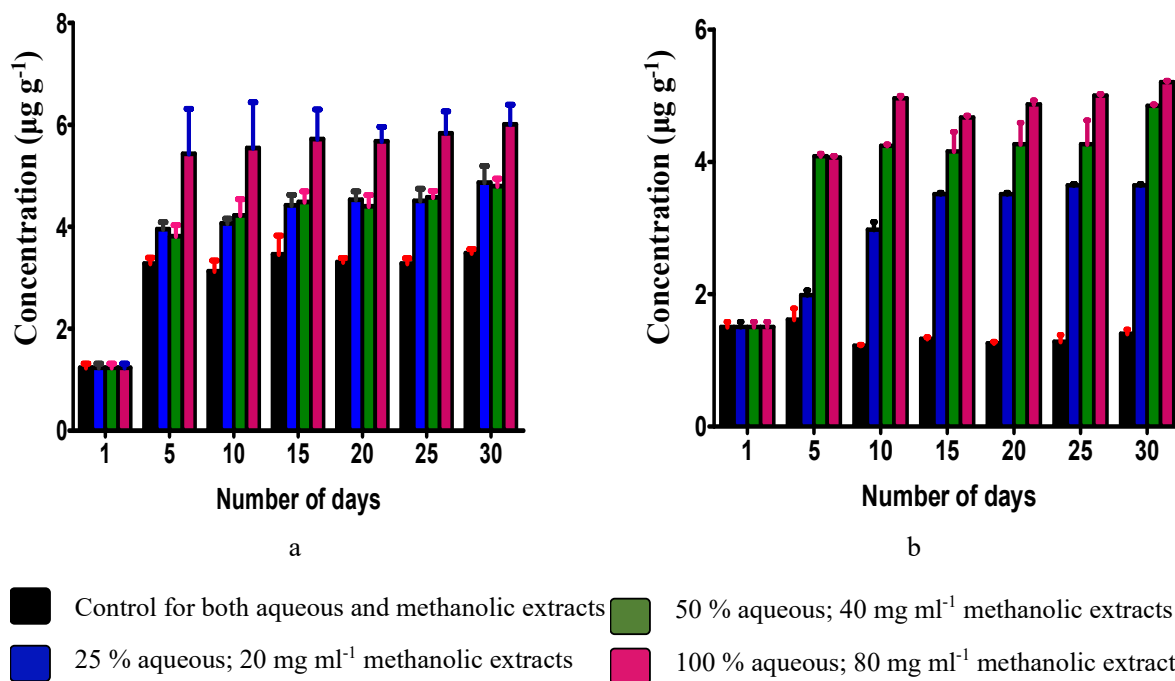


Figure 3. Effect of Varying Concentration of (a) Aqueous and (b) Methanolic Extracts of *Tithonia diversifolia* on the Proline Concentration in *Vigna unguiculata*

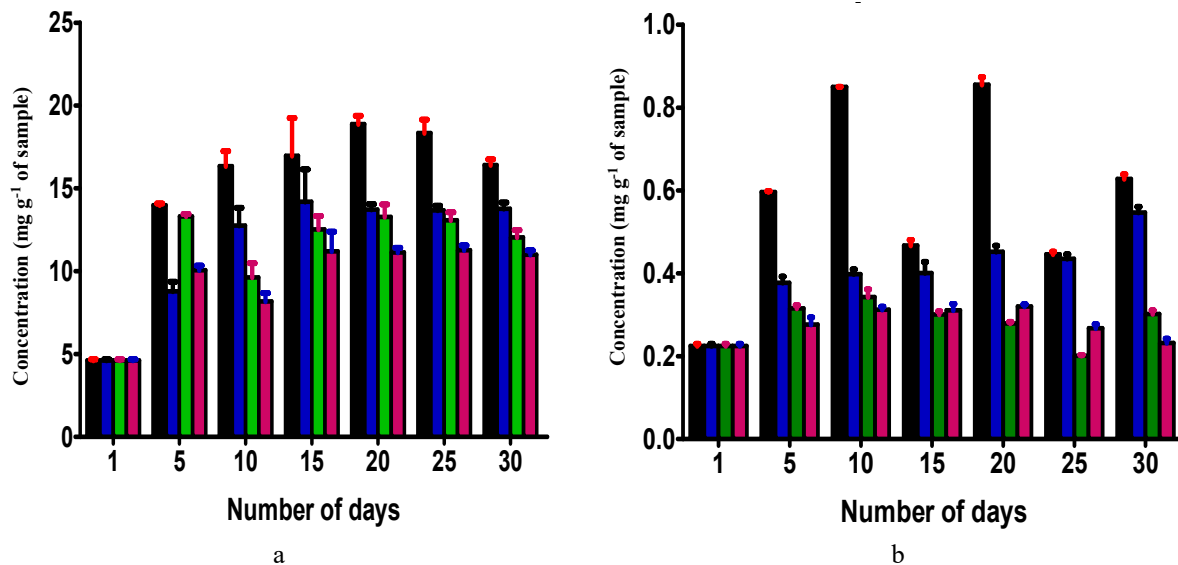


Figure 4. Effect of Varying Concentrations of (a) Aqueous and (b) Methanolic Extracts of *Tithonia diversifolia* on the Protein Concentration in *Vigna unguiculata*

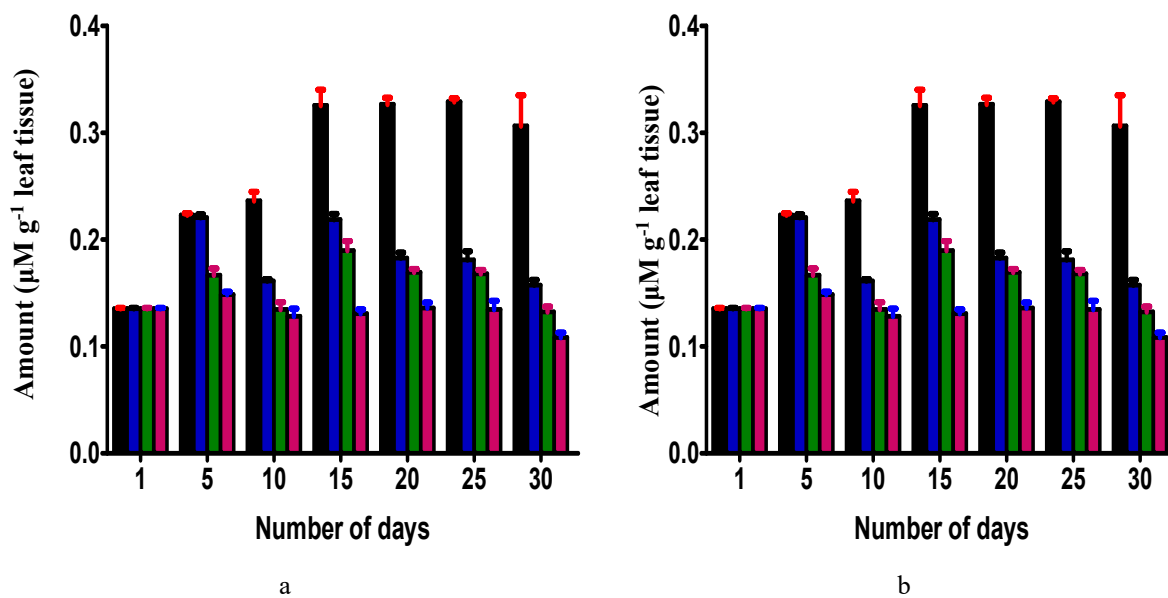


Figure 5. Effect of Varying Concentrations of (a) Aqueous and (b) Methanolic Extracts of *Tithonia diversifolia* on the Amount of Total Chlorophyll in the Leaf of *Vigna unguiculata*

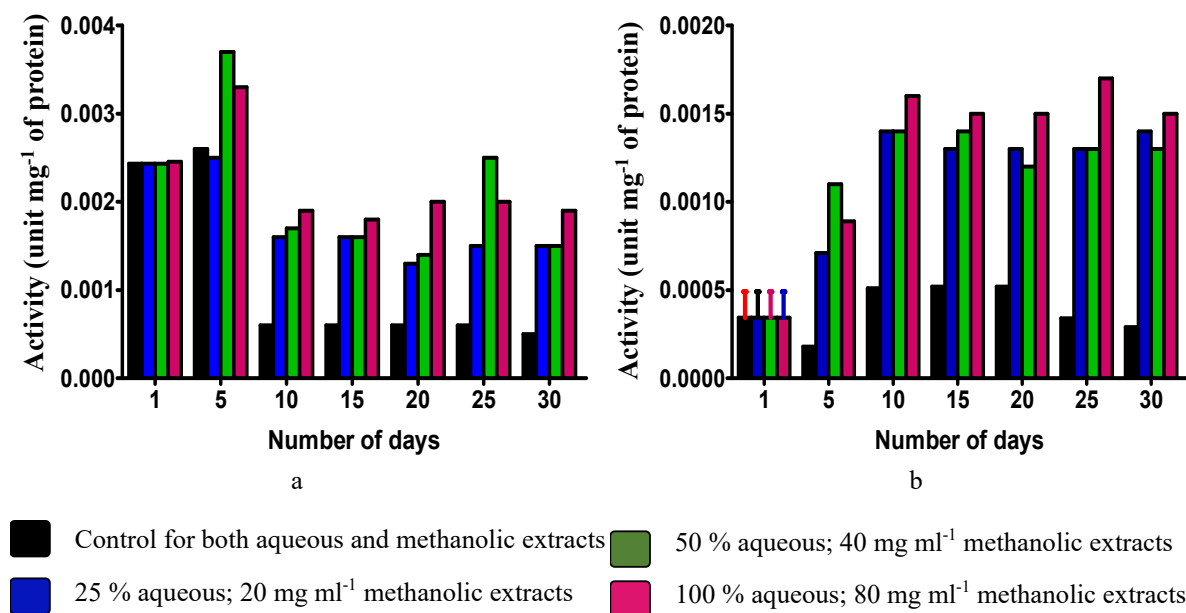


Figure 6. Effect of Varying Concentration of (a) Aqueous and (b) Methanolic Extracts of *Tithonia diversifolia* on the Catalase Activity in *Vigna unguiculata*

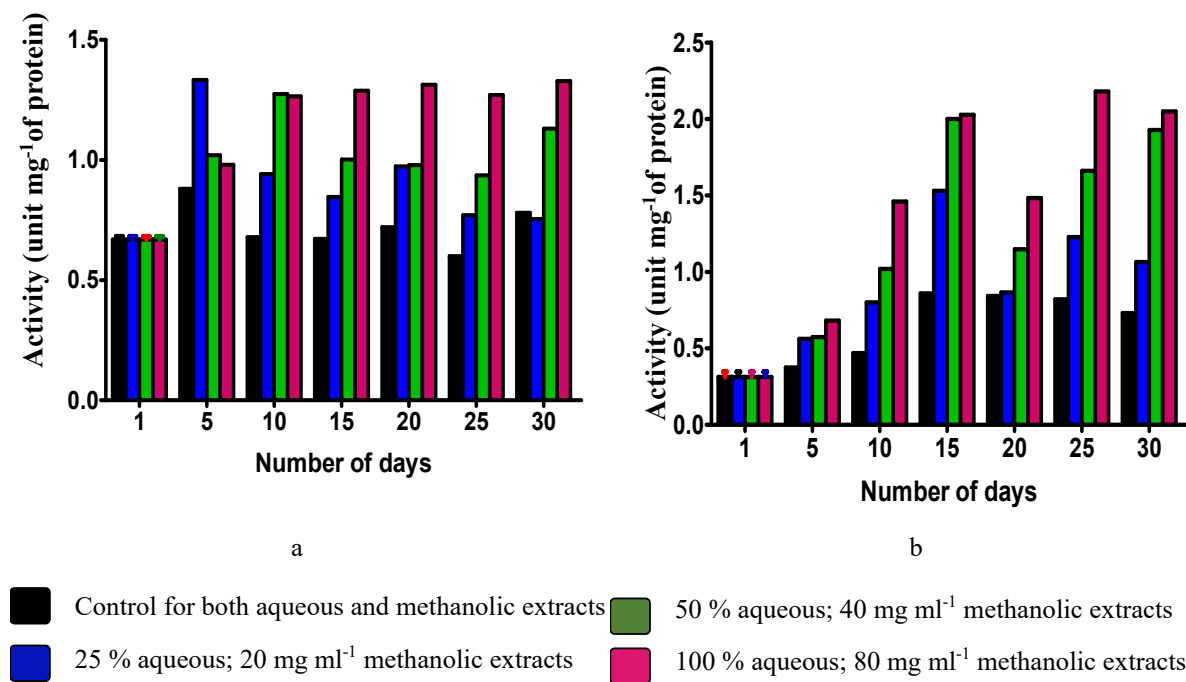


Figure 7. Effect of Varying Concentrations of (a) Aqueous and (b) Methanolic Extracts of *Tithonia diversifolia* on Superoxide Dismutase Activity in *Vigna unguiculata*

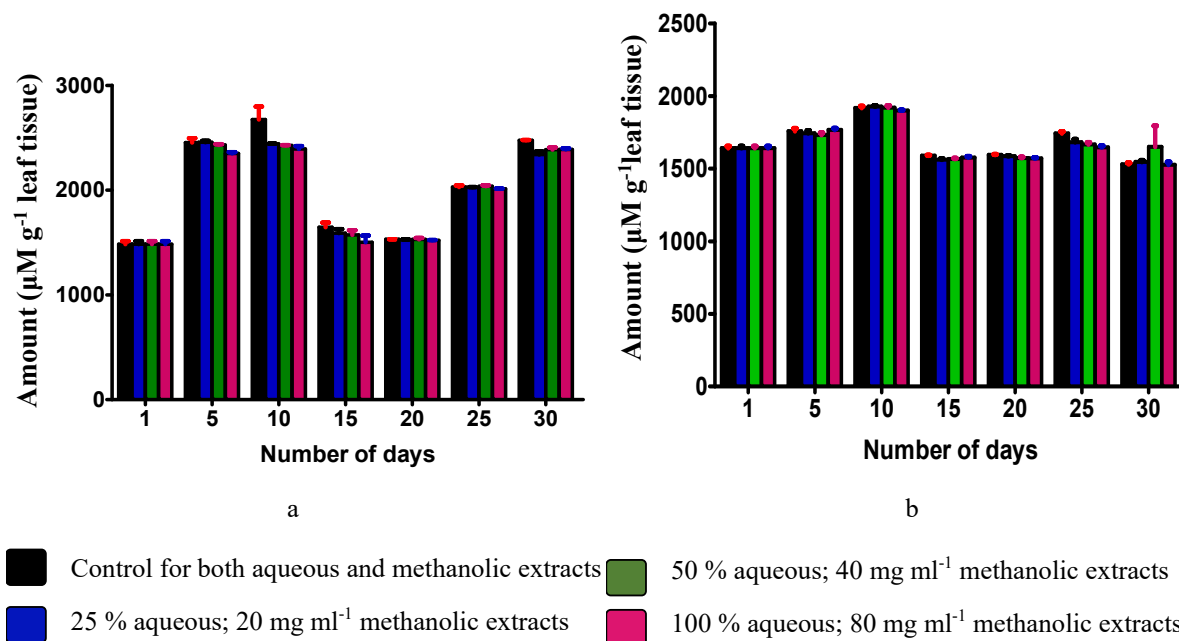


Figure 8. Effect of Varying Concentrations of (a) Aqueous and (b) Methanolic Extracts of *Tithonia diversifolia* on the on the Concentration of Total Soluble Sugar in *Vigna unguiculata*

4. Discussion

In this study, the effect of extracts of *T. diversifolia* on biochemical parameters of growth in *V. unguiculata* was investigated. The choice of biochemical parameters of growth was due to the fact that detrimental effects reported on allelopathic plants are secondary expressions of primary effects on metabolic processes. The aqueous and methanolic extracts of *T. diversifolia* were phytochemically screened for the presence of secondary metabolites; and the results showed the presence of secondary metabolites such as cardiac glycosides, flavonoids, saponins, alkaloids, and anthraquinones. Secondary metabolites such as phenols and quinones are known to pose toxicity to plants by formation of radicals (Testa, 1995). These radicals could affect membrane permeability, cause damage to DNA and proteins, and generate lipid peroxide signaling molecules (Yu et al., 2003). However, superoxide dismutase converts superoxide anion into hydrogen peroxide (H₂O₂), which can then be converted into harmless H₂O molecules in a reaction catalyzed by catalase. Several enzymes of the defense system increased tremendously during oxidative stress in order to avoid the damage caused by ROS (Foyer & Noctor, 2003). These defense enzymes act as stress markers. Their assay is an essential aspect in assessing stress responses in plants. The extracts of *T. diversifolia* induced stimulatory effect on the antioxidant system of *V. unguiculata*. This increase could be attributed to the ability of plants to improve scavenging system (Vaidyanathan et al., 2003). The marked increases in antioxidant enzymes (superoxide dismutase and catalase) have also been observed in other studies on modes of action of allelochemical such as, phenols (Yu et al., 2003). Also, Hegab (2005) and Ghareib et al. (2010) reported an increase in the activities of antioxidant enzymes in target plants treated with phenolic compounds.

The aqueous and methanolic extracts of *T. diversifolia* were found to inhibit the germination of *V. unguiculata* particularly at higher concentrations. The degree of inhibition increased as the concentration increased. For the methanolic extract, there was total inhibition at the highest concentration (80 mg ml⁻¹). According to Laosinwattanna et al. (2009), a number of previous studies have suggested that the degree of inhibition increases with increasing extract concentrations. One suggested mechanism for the inhibition of seed germination is the disruption of 'dark' or mitochondrial respiration (Podesta and Plaxton, 1994). Glycolysis, one of the phases of dark respiration is necessary to mobilize stored carbohydrates to provide the seed with the reducing power, ATP, and carbon products required for the biosynthesis of the roots and aerial parts of the emerging seedling (Weir et al., 2004). Several of phenolic compounds, such as vanillic, p-coumeric, p-hydroxybenzoic and protochatechuic acid, tested alone and in combinations, were able to inhibit the enzymatic activity of all or most of glycolytic enzymes (Muscolo et al., 2001). This suggests that the decrease in seed germination has a secondary effect on these enzymes.

During decomposition of decaying plant residues, phytotoxic compounds can be produced, released, transformed and destroyed simultaneously by microbial activities thus affecting plants (Blum et al., 1999). Changes over time of both composition and quantity of allelochemicals can either increase or decrease the effects of decomposing plant materials (An et al., 2001). In this study, the decomposed leaves of *T. diversifolia* significantly reduced the germination, root length and shoot length of *V. unguiculata*. This could be due to release of allelochemicals or toxins into the soil from the decaying residue by the action of microorganism during decomposition (Kumar et al., 2006). This result is in accordance with the work of Batish et al. (2002) in which germination, growth, and yield of four summer season crops (millet, sorghum, corn, and cluster bean) were decreased when grown in fields containing sunflower residues. Chou and Lin (1976) also reported that the aqueous extract of decomposing rice residues in soil contained five phenolics and the extract inhibited the radicle growth of lettuce and rice seeds and the growth of rice seedlings.

Several studies have revealed that phenolics acids can interfere with the production of chlorophylls. Many allelochemicals were reported to interfere with the chlorophyll production due to their interference with porphyrin containing compound (Kanchan & Jayachandra, 1980). Chlorophyll content of bean seedling treated with the extracts decreased significantly. This may be due to the interference of phenolics with the synthesis of porphyrin, a precursor of chlorophyll biosynthesis. Yang et al. (2002) studied the action of p-coumaric acids on the biosynthesis of porphyrin precursors and reported that their interference with Mg-chelatase affected the level of chlorophyll accumulation in rice seedlings. Also, Inderjit and Dakshini (1995) found that water soluble phenolics from *Pluchea lanceolata* influenced the chlorophyll content of the leaves of the test plants under greenhouse conditions.

Proline is an osmo-protectant in plants. Osmo-protectants are compounds synthesized by plants and other organisms in order to cope with osmotic stress (Yancey, 1994). In this study, the total proline content of cowpea seedling treated with the extracts increased significantly as the concentration of the extract increased. The accumulation of proline in *Vigna unguiculata* may be due to stress caused by the extracts of *Tithonia diversifolia*.

Biochemical mechanism is any chemical reaction or series of reactions, often enzyme-mediated, which result in physiological effect. Complex plant-plant and plant-microbe interactions in ecosystems and currently developing studies on molecular, cytological and physiological levels bring us to a better understanding of the processes involved in allelopathy. The results obtained from this study showed that the extracts (aqueous and methanolic) of *T. diversifolia* at different concentrations had effects on seed germination, seedling growth, protein concentration, proline content, amount of chlorophyll and the activities of some antioxidant enzymes (superoxide dismutase and catalase) in *V. unguiculata*.

The genotoxicity study on the onions treated with *T. diversifolia* extracts showed that there were no chromosomal aberrations and no effect on the mitotic index, suggesting that *T. diversifolia* is not genotoxic. This result is however, contrary to what has been reported for many plants that exhibit allelopathic effect, for example, Askin and Aslanturk in 2010 reported that the aqueous leaf extract of *Inula viscosa* induced significant amounts of chromosomal aberrations and micronucleus formation.

5. Conclusion

The allelochemicals in *Tithonia diversifolia* had significant effects on seed germination, seedling growth, and the biochemical parameters and growth of *Vigna unguiculata* and could possibly explain the mechanism of visible allelopathic effect observed in *Tithonia diversifolia* in the previous studies.

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