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Phytotoxic Effect of *Tithonia rotundifolia* (Miller) S.F.Blake on Chlorophyll and Protein Contents of *Vigna unguiculata* L. and *Zea mays* L.

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Abstract

A large number of plants impose inhibitory effects on the germination and growth of neighbouring or successional plants by releasing allelochemicals into the soil. This study investigated the phytotoxic effects of *Tithonia rotundifolia* (Miller) S.F.Blake on the chlorophyll and protein contents of *Vigna unguiculata* (L.) Walpers and *Zea mays* L.. This was with a view to determining the susceptibility of these crops to allelochemicals in the extracts prepared from *T. rotundifolia*. Seeds of the test plants were sown in pots filled with top humus soil. At two weeks, seedlings in each pot were thinned down to 10 seedlings per pot. Potted plants of the test crops were supplied with 400 ml of the appropriate water extracts while the control potted plants were supplied with 400 ml of water. Biochemical analyses were carried out according to standard methods. The data obtained were analysed by (ANOVA) to determine significant ($P< 0.05$) effects. The means were compared using Duncan Multiple Range Test. The chlorophyll a, chlorophyll b, total chlorophyll and protein contents in *V. unguiculata and Z. mays* and were significantly inhibited by the extract from *T. rotundifolia*. The inhibitory effects of these allelochemicals increased with concentration. The phytotoxic effect of *T. rotundifolia* was species dependent. It was concluded that the extract contains water-soluble allelochemicals which inhibited the biochemical parameters of the test crops. *T. rotundifolia* should be controlled where it grows in association with cultivated crops.

Keywords: phytotoxic, allelochemicals, *Tithonia rotundifolia,* water extracts, test crops

1. Introduction

Tithonia rotundifolia (Miller) S.F. Blake is a members of the family Asteraceae. In Nigeria, *T. rotundifolia* have colonized roadsides, waste places, fallow land and disturbed open spaces like abandoned construction sites etc. displacing traditional weedy species like *Chromolaena odorata and Panicum maximum* (Adebowale and Olorode, 2005). According to Tongma, *et al.* (1998), the plant associates with common crops like vegetables, cassava, yam, rice, sorghum, soyabean e.t.c. and becomes a dominant plant where it is present.

Cowpea (*Vigna unguiculata* (L.) Walpers) which belong to the family Fabaceae is economically significant legumes in the tropics. *V. unguiculata* is a staple food for the majority of the world population. Its grains are consumed by man as cheap plant protein since fish, meat, milk and egg proteins are fast disappearing (Alabi *et al*., 2003). Maize (*Zea mays* L.) is an annual grass belonging to the family Poacea. *Z. mays*. is one of the most important cereal crops growing in the world. It is used as food for human consumption as well as food grain for animals (Moussa, 2001).

Siyar *et al*. (2017) reported that weed incursion in cultivated fields is a serious biological problem, which causes considerable yield losses of economically important field crops through allelopathy and competitive interactions. Allelopathic interactions are mediated by secondary metabolites (allelochemicals) released through leaching, root exudation, volatilization and residue decomposition into the environment and affect growth and development in natural environments and agro-ecosystems (Cheema *et al.* 2013). Allelochemicals may be distributed broadly among organs such as seeds, flowers, pollen, leaves, stems, and roots, or sometimes found in just one or two such locations Zeng *et al*. 2008).

Masuda *et al.* (2002) observed that allelochemicals could inhibit the enzyme protoporphyrinogen oxidase and therefore lead to alteration in chlorophyll biosynthesis. They further stated that allelochemical effects on photosynthesis could also be the result of an alteration in chlorophyll degradation pathway and inhibition of carotenoid biosynthesis Yang *et al.* (2002) observed that chlorophyll biosynthesis of rice seedlings was inhibited by exogenously applied allelochemicals. The authors asserted that chlorophyll reduction must result in a decrease of photosynthesis efficiency. They also suggested that higher concentration of allelochemicals would inhibit physiological activities the more Zarnota, *et al.* (2003) found that some allelochemicals have potential phytotoxic activity as photosystem II inhibitor since chlorophyll molecules are the core component of pigment – protein complexes embedded in the photosynthetic membranes.

According to Maysa and Salama, (2009), the germination, shoot and root length, dry weight, water content, chlorophyll content, proteins, carbohydrates and proline of *Triticum Aestivum* were significantly inhibited by increasing the concentration of allelochemicals extracted from *Achillea santolina*. Saeid *et al*. (2010) found that allelochemicals decreased the amount of protein in wheat seedlings. Hussain *et al.* (2010) reported that ferulic acid and p-hydroxybenzoic acid significantly reduced the leaf protein contents of *Lactuca sativa.*

Considering the effects of *Tithonia* species on associated crops there is the need for studies on the allelopathic effects of this weed on important economic crop plants grown in Nigeria. Therefore, the objective of the research was to determine the effects of water extracts of fresh shoots of *T. rotundifolia* on the chlorophyll and protein accumulation of *V. unguiculata and Z. mays*

2. Materials and Methods

2.1 Plant Materials and Extraction

The seeds of V*igna unguiculata* and *Zea mays* were collected from IITA (International Institute of Tropical Agriculture) Ibadan. *T. rotundifolia* seeds were collected Staff Quarter of Obafemi Awolowo University (O. A. U) Ile-Ife, Osun State, Nigeria. Plastic pots (25 cm diameter x 22 cm height) with four holes perforated at the bottom for good drainage were filled almost to the brim with top humus soil. The seeds of *T. rotundifolia* were sown in each of the pots and watered with 400 ml of tap water every morning. Extraction procedures was carried out according to the modified method of Qasem and Abu – Irmaileh (1985). Fresh plants of *T.rotundifolia* were harvested before flowering and separated into shoots and roots. 250 g of the fresh shoots were cut into small chips of about four centimeter lengths and finely ground with a mortar and pestle. The ground plant material was soaked in two litres of water for twelve hours. The solution was filtered through cheese cloth to remove debris and then filtered through Whatman No 1 filter paper. This extract solution (100%) was diluted appropriately with water to give 75%, 50%, and 25% concentrations of the aqueous extracts while distilled water served as control

2.2 Experimental Design and Treatment

Plastic pots (25 cm diameter x 22 cm height) with four holes perforated at the bottom for good drainage were filled almost to the brim with top humus soil. Seeds of the test plants were sown at equal distance in the pots and watered with 400 ml of tap water every morning. At two weeks, seedlings in each pot were thinned down to 10 seedlings per pot. Thereafter, the pots in the control regime were supplied with water daily while the pots belonging to the different treatments were supplied with either the appropriate water extracts (100 FWE,75% FWE 50% FWE 25% FWE) daily in same quantity. Treatments were arranged in a randomized complete block design with five replications.

2.3 Determination of Chlorophyll and Protein Content

Chlorophyll contents were determined using the method of Comb *et al*. (1985). Plants were separated into shoot and root and then chlorophyll was extracted from the shoot. The shoot was cut into small chips and placed in a mortar. A pinch of sodium bicarbonate was added to the shoot in the mortar to prevent degradation of chlorophyll to phaeophytin and then the shoot was then ground in 80% (v/v) acetone. The brei was filtered through a Whaman No 1 filter paper and absorbance of the acetone filtrate was determined using a spectrophotometer at wavelength 647nm and 664nm.

Chlorophyll a, chlorophyll b and total chlorophyll were determined using the formulae below.

Chlorophyll a = $13.19A_{664} - 2.57A_{647}$ (µg/g)

Chlorophyll b = $22.10A_{647} - 5.26A_{664}$ (µg/g)

Total chlorophyll = $7.93A_{644} + 19.53 A_{647}$ (µg/g)

Where A_{647} is absorbance at 647 nm wavelength, A_{664} is absorbance at 664 nm wavelength

Total protein concentration was determined using the technique of Lowry *et al.* (1951).

2.4 Statistical Analysis

The results were analyzed statistically with the use of one-way analysis of variance (ANOVA) to determine significant ($P< 0.05$) effects. The means were compared using Duncan Multiple Range Test (DMRT)

3. Results

Tables 1a &1b show the effects of fresh shoot water extracts (FWE) of *T. rotundifolia* on *V. unguiculata*. Results indicated that chlorophyll a, chlorophyll b, total chlorophyll in the control shoots of *V. unguiculata* were significantly different at $p < 0.05$ and higher than those of the shoot of seedlings treated with the different extracts throughout the period of the experiment. It was observed that the highest concentration 100% FWE inhibited the total chlorophyll content by 27% while the 25% extract reduced the total chlorophyll by 13%. The extent of the inhibition of chlorophyll a, chlorophyll b, total chlorophyll by these extracts was observed to follows this order 100% >75% >50% >25% i.e The allelopathic retardatory effect on chlorophyll contents observed were extract concentration dependent

The protein contents of the extract treated plants were significantly ($P < 0.05$) lower than that of the control plants. The protein content decreased from 3.20 (µg) in the control plants to 1.07 (µg) in the plants treated with 100% FWE. The 25 % FWE treated plants had a protein content that was decreased by 13% when compared with the control plants. Protein content showed significant decrease by increasing the extract concentration. The effect of fresh shoot extracts of *T. rotundifolia* on *Z. mays* is shown in Tables 2a & 2b. Chlorophyll a, chlorophyll b and total chlorophyll in the extracts treated *Z. mays* were significantly lower than that of the control plants. The total chlorophyll of the 100% FWE plants was reduced by 26% compared to the control plants. The potency of allelochemicals in the extracts was dependent on the concentration of the extracts in most cases. The extent of the inhibition of chlorophyll a, chlorophyll b, total chlorophyll by these extracts was observed to follows this order 100%>75% >50% >25% i.e The allelopathic retardatory effect observed was extract concentration dependent. Protein content of the control plant was significantly ($P < 0.05$) higher than those of 100%FWE. The plants treated with highest extract concentration (100%) of *T. totundifolia* showed 42% decrease with respect to control. The protein contents of *Z. mays* in 75% FWE, 50% FWE and 25% FWE were not significantly different from that of the control.

Table 1a. Effect of fresh shoot water extracts (FWE) of *T. rotundifolia* on chlorophyll a and chlorophyll b contents of *V. unguiculata*

Chlorophyll a (µg/g)	Week 1	Wewek2	Week 3
Control	381.00 ±.32 [a]	410.60 ±3.03[a]	480.47 ±1.60[a]
100 % FWE	200.32 ±.81[b]	301.12 ±.97 [b]	310.03 ±2.73[b]
75% FWE	210.33 ±.21[c]	320.30 ±66 [c]	340.20 ±2.10[c]
50% FWE	280.60 ±1.9 [d]	348.90 ±.84 [d]	399.24 ±4.33[d]
25% FWE	280.98 ±1.4 [d]	380.82 ±1.7 [e]	410.26 ±2.65[e]
Chlorophyll b (µg/g)			
Control	211.38 ±.75 [a]	285.60 ±1.70 [a]	313.76 ±1.61[a]
100 % FWE	80.31 ±1.24 [b]	71.60 ±.50 [b]	199.47 ±3.03[b]
75% FWE	179.50 ±.45 [c]	140.04 ±.63 [c]	251.26 ±2.26[c]
50% FWE	184.40 ±1.21[d]	241.20 ±.48 [d]	281.46 ±6.47[d]
25% FWE	201.20 ±2.25 [e]	211.82 ±1.2 [e]	301.40 ±4.24[e]

FWE: Fresh shoot water extract treatment. Figures on the same column followed by different letters show significant differences with each other at $p < 0.05$ according to DMRT

Table 1b. Effect of fresh shoot water extracts (FWE) of *T. rotundifolia* on total chlorophyll and protein contents of *V. unguiculata*

Total Chlorophyll (µg/g)	Week 1	Wewek2	Week 3
Control	579.10 ±5.46 [a]	673.20 ±2.20 [a]	846.69 ±1.58 [a]
100 % FWE	305.29 ±.66 [b]	384.80 ±2.57 [b]	620.57 ±7.84 [b]
75% FWE	387.74 ±6.51 [c]	471.21 ±1.20 [c]	620.70 ±3.22 [b]
50% FWE	457.66 ±1.50 [d]	565.60 ±1.07 [d]	674.08 ±1.94 [c]
25% FWE	473.40 ±1.72 [e]	685.42 ±7.51 [e]	747.50 ±1.43 [d]
Protein content (µg)			
Control	2.61 ±.03 [a]	2.64 ±.07 [a]	3.20 ±.03 [a]
100 % FWE	1.09 ±.02 [b]	1.17 ±.03 [b]	1.07 ±.11 [b]
75% FWE	1.16 ±.04 [b d]	1.31 ±.14 [b d]	1.28 ±.01 [c]
50% FWE	1.22 ±.02 [c d]	1.61 ±.05 [c]	2.77 ±.02 [d]
25% FWE	1.27 ±.03 [c]	1.47 ±.06 [c d]	2.84 ±.02 [d]

FWE: Fresh shoot water extract treatment. Figures on the same column followed by different letters show significant differences with each other at $p < 0.05$ according to DMRT

Table 2a. Effect of fresh shoot water extracts (FWE) of *T. rotundifolia* on chlorophyll a and chlorophyll b contents of *Z. mays*

Chlorophyll a (µg/g)	Week 1	Wewek2	Week 3
Control	485.44 ± 2.22 [a]	553.52 ± 3.37 [a]	599.92 ± 3.32 [a]
100 % FWE	301.27 ± .70 [b]	403.20 ± 2.17 [b]	499.36 ± 2 .57 [b]
75% FWE	361.00 ± 1.00 [c]	445.80 ± 2.03 [c]	554.72 ± 4.45 [c]
50% FWE	461.20 ± 2.80 [d]	497.76 ± 2.21 [d]	580.14 ±4.11 [d]
25% FWE	497.26 ± 2.61 [e]	558.34 ± 3.51 [e]	560.06 ± 5.48 [c]
Chlorophyll b (µg/g)			
Control	400.60 ± .40 [a]	422.40 ± 2.54 [a]	400.02 ± 4.11 [a]
100 % FWE	145.60 ± .40 [b]	308.48 ± .93 [b]	310.29 ± 3.17 [b]
75% FWE	308.60 ± .60 [c]	320.80 ± .86 [c]	420.00 ± 3.16 [c]
50% FWE	353.15 ± 2.5 [d]	482.66 ± 2.26 [d]	400.14 ± 3.79 [a]
25% FWE	470.20 ± 1.98 e	404.40 ± 1.08 [e]	461.68 ± 2.76 [d]

FWE: Fresh shoot water extract treatment. Figures on the same column followed by different letters show significant differences with each other at $p < 0.05$ according to DMRT

Table 2b. Effect of fresh shoot water extracts (FWE) of *T. rotundifolia* on total chlorophyll and protein contents of *Z. mays*

Total Chlorophyll (µg/g)	Week 1	Wewek2	Week 3
Control	867.20 ±2.95 [a]	927.60 ±2.50 [a]	987.88 ±3.33 [a]
100 % FWE	424.00 ±8.46 [b]	720.80 ±.66 [b]	730.12 ±3.16 [b]
75% FWE	662.82 ±3.59 [c]	722.10 ±1.10 [b]	962.12 ±2.52 [c]
50% FWE	819.40 ±2.80 [d]	942.40 ±5.30 [c]	955.20 ±4.14 [c]
25% FWE	931.60 ±2.6 [e]	945.16 ±4.29 [c]	978.40 ±3.25 [a]
Protein content (µg)			
Control	1.05 ±0.03 [a]	1.31 ±.02 [a]	1.39 ±0.03 [a]
100 % FWE	0.49 ±0.01 [b]	0.55 ±.03 [b]	0.80 ±.03 [b]
75% FWE	0.88 ±0 .01 [c]	1.25 ±.02 [a]	1.38 ±0.09 [a]
50% FWE	0.87 ±0.04 [c]	1.34 ±.02 [a]	1.56 ±.14 [a]
25% FWE	0.92 ±0.03 [c]	1.38 ±.11 [a]	1.61 ±.06 [a]

FWE: Fresh shoot water extract treatment. Figures on the same column followed by different letters show significant differences with each other at $p < 0.05$ according to DMRT

4. Discussion

Chlorophyll a, chlorophyll b and total chlorophyll contents in the shoots of *V. unguiculata* and *Z. mays* were inhibited by the application of the different extracts. This result correlates with the findings of some earlier workers who reported that extracts from allelopathic plants were capable of impairing chlorophyll synthesis thereby reducing chlorophyll accumulation. For example, Nitesh and Ambika (2016) reported a was significant reduction in chlorophyll contents of wheat at high concentrations of the weed extracts. Sypek *et al.* (2015) stated that increasing concentrations of aqueous extracts of peppermint caused a decrease of chlorophyll a and an increase of chlorophyll b content of *Helianthus annuus* L. According to Sonbeer *et al.* (2017), relative leaf water content, total leaf chlorophyll content and leaf N P K content of French bean were reduced by the aqueous extract of Jatropha. The chlorophyll content of the *A. pedunculata* leaves decreased with an increase in the aqueous leaf extracts concentration of the four shrubs (Wang *et al.*, 2018)

Yang *et al.* (2002) was of the opinion that allelochemicals may reduce chlorophyll accumulation in three ways namely: the inhibition of chlorophyll biosynthesis; the stimulation of chlorophyll degradation or both. The allelochemicals present in all the aqueous extracts must have inhibited chlorophyll accumulation primarily through reduction in chlorophyll synthesis or stimulation of chlorophyll degradation. A consequent reduction in net photosynthesis of the plants would be expected. That is, such inhibition of chlorophyll accumulation in the plants would be expected to naturally reduce photosynthesis and ultimately the total plant growth.

The protein contents in the shoots of *V. unguiculata* and *Z. mays* were inhibited by the application of the different extracts. The reduction in protein contents in the extract treated plants may be attributed to the effect of allelochemicals on DNA replication or transformation by intercalation with nucleic acids by ionic bonding with their negatively charged phosphate groups or the accumulation of phenolic glycine that interferes with the cytoplasmic ribosomes and production of RNA, which in turn inhibited protein synthesis (Hegab and Ghareib, 2010). Ramakrishnan *et al.* (2014) similarly reported that leaf leachates of *Gmelina arborea* inhibited protein content in green gram, red gram, black gram, and chickpea. According to Saeid (2014), there was a significant reduction in the amount of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and protein in *Capsicum annuum* L. in response to allelochemical stress of aqueous leachate of *Achillea biebersteinii* Higher and lower concentration of extracts inhibited the protein contents in *V. unguiculata* whereas only higher concentration (100% FWE) reduced protein contents in *Z. mays* compared with control. This indicated that the response of plants to allelochemicals toxicity was dependent on plant species i.e. there was an interspecific differential response to allelochemicals toxicity. This was consistent with the work of Maharjan *et al.* (2007) who stated that sensitivity to allelochemicals and extent of inhibition varied with species and organs of the test species.

5. Conclusion

The present study suggested that the aqueous extract prepared from *T. rotundifolia* inhibited the chlorophyll and protein contents of *Vigna unguiculata* (L.) Walpers and *Zea mays* L Therefore, the extracts contain water-soluble allelochemicals which can suppress the metabolic process of the test crops.

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