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Changes in Chlorophyll and Protein Contents of *Vigna unguiculata*, *Glycine max*, *Zea mays* and *Sorghum bicolor* Raised in Soil Incorporated with the Shoots of *Tithonia rotundifolia*

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Authors' contributions

This work was carried out in collaboration between both authors. Author OJI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OJI and OOO managed the analyses of the study. Author OOO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Tithonia. rotundifolia is an allelopathic weed that grows in association with cultivated crops in Nigeria. Allelopathy is a phenomenon of plant releasing allelochemicals into the environment that can inhibit or stimulate the growth of other plants and microorganisms. This study aimed to evaluate the effects of soil incorporated with the fresh shoots of *T. rotundifolia* on chlorophyll and protein accumulation of *Vigna unguiculata* L. *Glycine max* L., *Zea mays* L. and *Sorghum bicolor* L. 250 g of fresh shoots of *T. rotundifolia* were worked into each plot of 2 m^2 dimension and the test crops were sown in the plots. Plots with no *T. rotundifolia* shoots were included as control plots. The experiment was performed in completely randomized block design (CRBD). The parameters measured were chlorophyll a, chlorophyll b total chlorophyll and protein content. The results showed a stimulation in chlorophyll contents in the test crops except inhibition in chl b and total chlorophyll in *Zea mays* L. Also, there was a stimulation of protein in *Zea mays* L. and *Sorghum bicolor* L. The study suggests that incorporation of shoots of *Tithonia rotundifolia* could imparts stimulatory allelopathic effects.

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1. INTRODUCTION

Tithonia rotundifolia (Miller) S.F. Blake is a members of the family Asteraceae. 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 [1]. Ayeni et al. [2] stated that *Tithonia* species are aggressive weeds with high invasive capacity and the ability to compete successfully with agricultural crops.

Cowpea (*Vigna unguiculata* (L.) Walpers) and Soybean (*Glycine max* (L.) Merr.) which belong to the family Fabaceae are economically significant legumes. Maize (*Zea mays* L.) and Sorghum (*Sorghum bicolor* (L.) Moench) are annual grasses belonging to the family Poacea. *Z. mays* L. is one of the most important cereal crops. *S. bicolor* (L.) Moench is a drought resistant cereal important for grain, forage and bioethanol production [3].

Allelopathy can be defined as the effects of one plant species on another plant species (mostly harmful effects) through the release of chemical compounds (allelochemicals) that escape into the environment [4]. Allelopathic interactions are mediated by secondary metabolites, released through leaching, root exudation, volatilization and residue decomposition into the environment and affect growth and development in natural environments and agro-ecosystems [5]. Chemical exudates from some plants to the environment have been reported as causative agents of allelopathy on growth of neighboring plants and thus affecting normal growth in their natural environment [6]. The allelopathic potential of some plants through the release of allelochemicals has either deleterious or beneficial effects on other plants associated in same locality [7]. *Tithonia rotundifolia* releases allelochemicals from the shoots and roots that have inhibitory or stimulatory effects on other plant species. The allelochemicals detected in the aqueous extracts of *Tithonia* sp were phenols, flavonoids, tannins, saponins and alkaloids. It is known that during decomposition of decaying plant residues, phytotoxic compounds can be produced, released, transformed and destroyed simultaneously by microbial activities [8], thus affecting both plants and microorganisms. According to Inderjit et al. [9], the bioactive concentration of the allelochemicals in soils is determined through sorption, fixation, leaching, chemical and microbial degradation. Soil chemicals, physical and biological characteristics to a great extent are responsible for detoxification or further enhancement of the allelopathic activities of the plant diffusates [10]. The decrease in chlorophyll content due to allelopathic effects has been reported [4,11,12,13,14]. Rice [4] suggested that some allelopathic compounds might interfere with the synthesis of porphyrin which is a precursor of chlorophyll thereby leading to a reduction of chlorophyll accumulation. The author was of the opinion that a reduction in chlorophyll accumulation would result in impaired photosynthesis and finally diminished total plant growth. Masura et al. [15] 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. Many studies have shown the effects of extract on protein contents of plants [16,17,18,19]. Therefore, the objective of this study was to determine the effects of soil incorporated with the fresh shoots of *T. rotundifolia* on chlorophyll and protein accumulation of *Vigna unguiculata* L. *Glycine max* L., *Zea mays* L. and *Sorghum bicolor* L.

2. MATERIALS AND METHODS

The soil was tilled and divided into square plots and 250 g of chopped fresh shoots of *T. rotundifolia* were worked into each plot. The test crops were sown in the plots. Plots with no *T. rotundifolia* shoots were included as control plots. The experiment was kept weed-free by hand weeding throughout the growing period. The plants were harvested two weeks after sowing on the treatment plots. Thereafter, harvesting of the plants was on a weekly interval until the end of the experiment.

2.1 Determination of Chlorophyll and Protein Content

Chlorophyll contents were determined using the method of Comb et al. [20]. 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. [21].

2.2 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

The chlorophyll a content of *V. unguiculata* planted on soil with shoots of *T. rotundifolia* (SIS) was higher than that of the plants in the control up to the fourth week of harvest (Fig. 1). Results of the ANOVA showed that there was significant difference between the chlorophyll a content in the control plants and that of the plants in the SIS regime at $P < 0.05$. The level of chlorophyll b in the control *V. unguiculata* plants increased from the beginning to the end of the experiment while that of the plants in the SIS regime followed a zig zag pattern throughout the duration of the experiment. As a result of this, the chlorophyll b content of the plants in the SIS regime was higher than that of the plants in the control in weeks 2, 4 and 6 of the experiment (Fig. 1). The total chlorophyll content of the control *V. unguiculata plants* followed a zig zag pattern from the beginning to the end of the experiment but remained lower than that of the plants in the SIS regime from the first week to the fourth week of harvest (Fig. 1).

The chlorophyll a content of the SIS *G. max* was higher than that of the plants in the control throughout the period of the experiment (Fig. 2). The chlorophyll b content of the SIS plants was higher than that of the plants in the control in most weeks of the experiment (Fig. 2). The total chlorophyll content of the SIS plants increased and was higher than that of the control plants from the beginning of the experiment up to week three. Thereafter, it decreased the following week and then finally increased until the end of the experiment (Fig. 2). Fig. 3 shows the effect of the soil incorporated with the shoots of *T. rotundifolia* on the chlorophyll contents of *Z. mays*. The accumulation of chlorophyll a in the SIS plants was higher than that of the plants in the control plants as from the third week until the end of the experiment. The chlorophyll b and total chlorophyll contents in the control plants were higher than that of the plants in the SIS regime in most part of the experiment (Fig. 3). The level of chlorophyll a, b and total chlorophyll content of the *S. bicolor* treated plant was higher than that of the control plants in most part of the experiment and this was found to be statistically significant at $p < 0.05$ (Fig. 4).

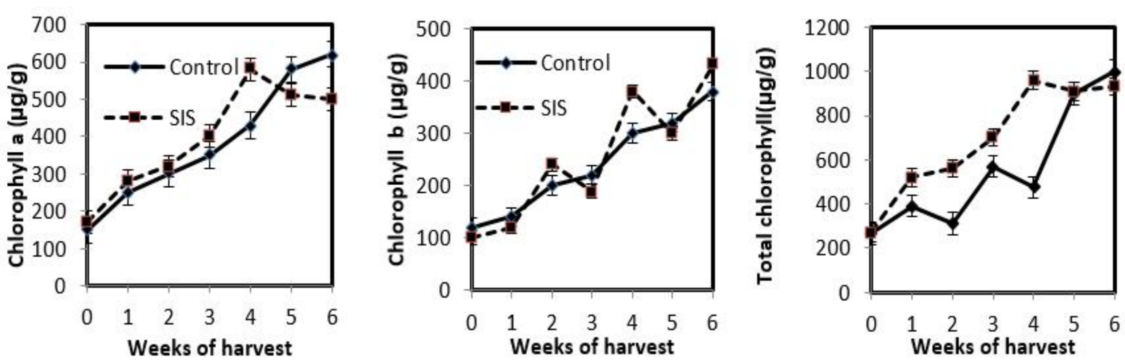

Fig. 1. Changes in chlorophyll a, b and total chlorophyll content of *V. unguiculata* raised in soil incorporated with the shoots of *T. rotundifolia*
SIS = soil incorporated with shoots of T. rotundifolia

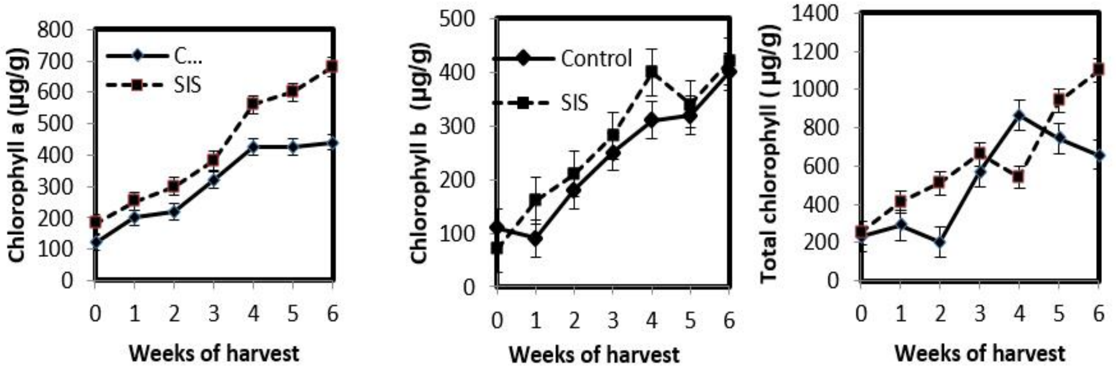

Fig. 2. Shows the effect of soil incorporated with the shoot of *T. rotundifolia* on the chlorophyll a, b and total chlorophyll content of *G. max*

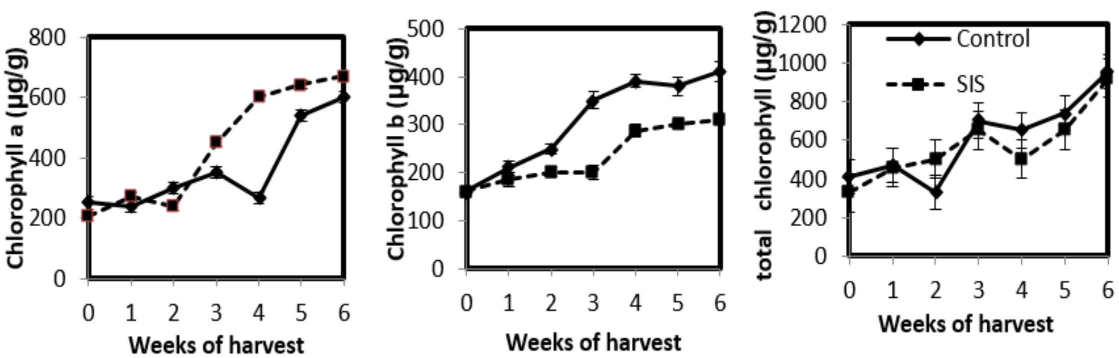

Fig. 3. Effect of the soil incorporated with the shoots of *T. rotundifolia* on the chlorophyll a, b and total chlorophyll content of *Z. mays*

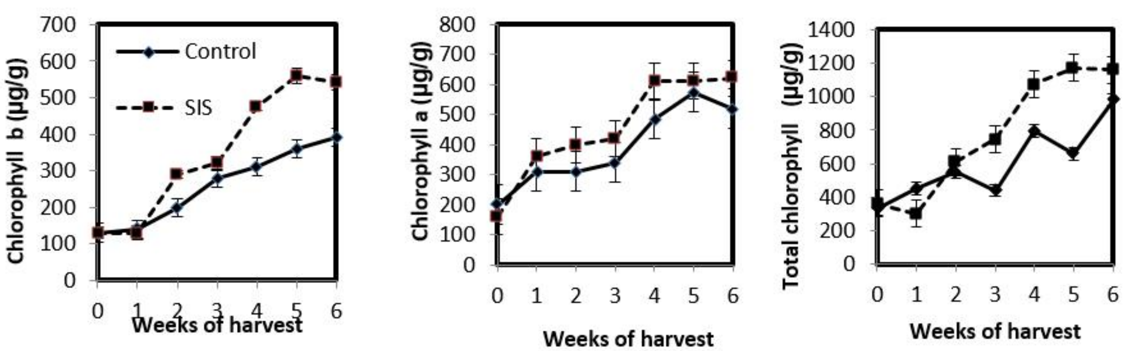

Fig. 4. Effect of the soil incorporated with the shoots of *T. rotundifolia* on the chlorophyll a, b, and total chlorophyll content of *S. bicolor*

SIS = Soil incorporated with shoots of T. rotundifolia

The protein content of the control *V. unguiculata* was higher than that of the SIS plants throughout the period of the experiment. Significant difference was observed between the protein content of the control plants and that of the SIS plants at $p < 0.05$ (Fig. 5). The protein content in the control *G. max* increased from the beginning of the experiment to week two and then decreased the following week. Thereafter, the protein content of these plants increased until the end of the experiment. In the case of the SIS plants, the protein content was initially higher

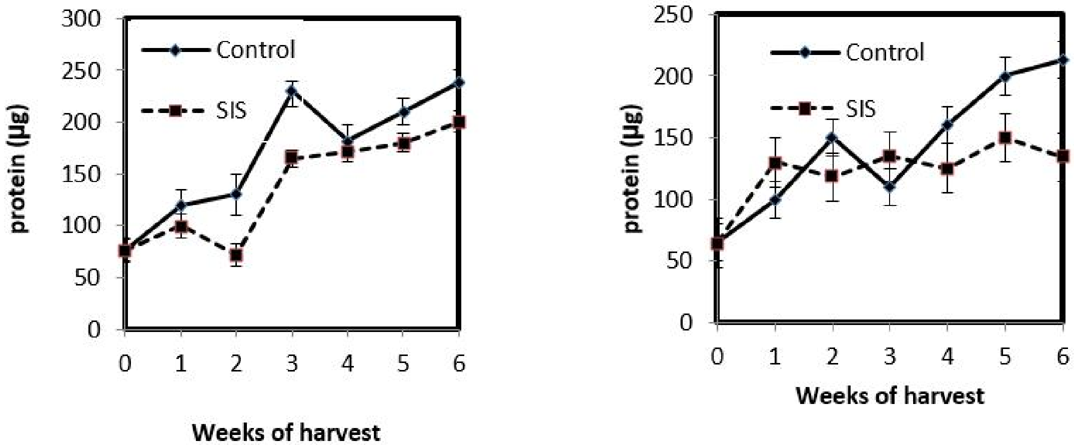

Fig. 5. Effect of soil incorporated with the shoots of *T. rotundifolia* on the protein content of *V. unguiculata and G. max*

a. V. unguiculata b. G. max

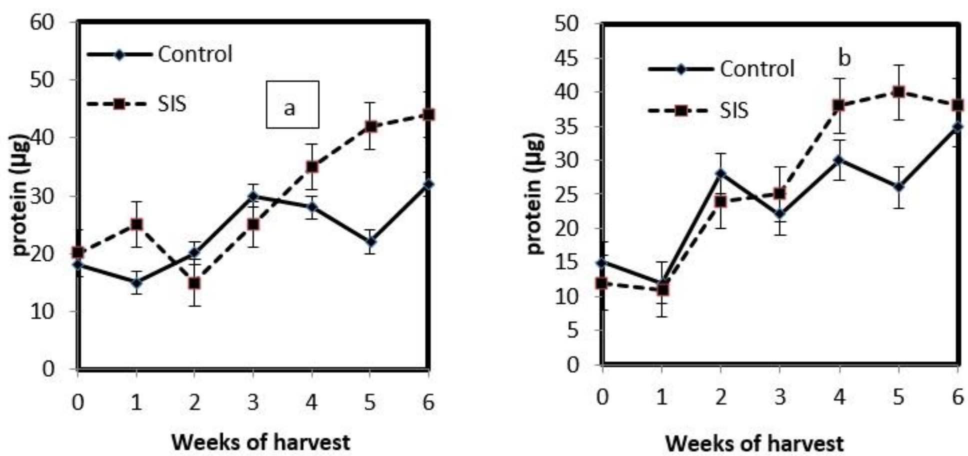

Fig. 6. Effect of the soil incorporated with the shoots of *T. rotundifolia* on the protein content of *Z. mays* and *S. bicolor*

a. Z. mays b. S. bicolor

than that of the control plants and then followed a zig zag pattern such that it became lower than that of the control plants in the last three weeks of the experiment. Significant difference was observed between the protein content of the control plants and that of the SIS plants at $p < 0.05$ (Fig. 5). The protein level of the SIS *Z. mays* and *S. bicolor* was significantly ($P < 0.05$) higher than that of the control plants during most weeks of the experiment (Fig. 6).

4. DISCUSSION AND CONCLUSION

Unlike allelochemicals obtained directly by extraction from living organs or tree litter, allelochemicals released from decomposed leaf litter are influenced by soil; thus their concentration, composition, structure, and activity might be extremely different. The important circumstances in which allelopathic effects appear occur when allelochemicals reach the recipient plant in their active structure and at their effective concentration, thus extracts of litter and decomposed litter (or the decomposed medium) often show different allelopathic effects [22]. According to Inderjit [9], soil microorganisms have important modifying effects on allelochemicals, and are able to decompose some of these chemicals into inactive substances. Lankau [23] was of the opinion that low concentration of allelochemicals in the soil may be as a result of the allelochemicals being decomposed by soil microbes.

In this study, accumulation chlorophyll in *Vigna unguiculata* L. *Glycine max* L. and *Sorghum bicolor* L. grown in the soil into which the shoot of *T. rotundifolia* had earlier been incorporated were all enhanced significantly compared to the control plants. It was evident that the incorporated shoot materials contained allelochemicals which on interacting with some microbes and other soil factors became stimulatory to the growth of the test plants. The bioactive concentration of the allelochemicals in the incorporated shoots might have been decreased due to microbial degradation and decomposition in the soil. Hence the detoxification of the plant residue and the resultant stimulatory allelopathic effects observed in this study. This was consistent with the observation of Einhellig et al. [24] who stated that allelochemicals have to be present above a threshold concentration for impact. He was of the opinion that some plants processes might be stimulated below this threshold. This result was also consistent with the finding of Qasem [25] who reported that the incorporation of *A. gracilis* residues stimulated plant height and increased wheat yield. The protein level of the SIS *Z. mays* and *S. bicolor* was significantly ($P < 0.05$) higher than that of the control plants during most weeks of the experiment. Protein content of *Rumex dentatus* has been reported to be stimulated by lower concentration root extracts or allelochemicals [17]. This stimulation was correlated with stimulation in nucleic acid content. According to Baziramakenga et al. [26], allelochemical increased incorporation of 3S S methionine into protein. The stimulation of the chlorophyll and protein contents of some of the test crops in this study indicated that *T. diversifolia* could be used as green manure.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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