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Biochemical Parameters of *Glycine Max* (L.) and *Sorghum bicolor* (L.) as Affected by the Water and Methanol Extracts of *TithoniaRotundifolia*

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ABSTRACT:
Glycine max (L.) Merr. and *Sorghum bicolor* (L.) Moench are economically significant crops in the tropics. It has been found out that most of the total crop losses are as a result of allelopathic interaction and weed competition. This study investigated the allelopathic effects of *Tithoniarotundifolia* on the chlorophyll and protein contents of *Glycine max* L. and *Sorghum bicolor* L. 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. Computer software SPSS was used for statistical analysis. The chlorophyll a,chlorophyll b, total chlorophyll and protein contents in *G. max* L. and *S. bicolor* L. were significantly inhibited by the extracts from the donor plant. The extent of the inhibition of these biochemical parameters by the water and methanolic extracts followed this order: 100% > 75% > 50% > 25%. There was an interspecific differential response to the toxicity of allelochemicals. It was concluded that *T. rotundifolia* had allelopathic potential which reduced chlorophyll and protein contents of the test crops. This study showed that allelochemicals in*T.rotundifolia*were soluble and better extracted by the organic methanolic solvent than water as the methanolic extract being more phytotoxic than the water extracts had a more pronounced retardatory effects on the chlorophyll and protein contents of the test crops.

KEY WORDS: Phytotoxic, allelochemicals, *Tithoniarotundifolia*, water extracts,methanolic extract, test crops

I. INTRODUCTION

Allelopathy is a harmful or beneficial interaction between plants, accomplished through the release of chemical substances called allelochemical into the environment. Allelopathic interactions have been known to occur in different groups of plants like algae, lichens, annual and perennial weeds (Rice,2013). Allelochemicals have direct and indirect useful or harmful effects on growth and physiological parameters of received plants (Rice,1984; Oracz*et al*., 2007). The effects of allelochemicals action are detected at molecular, structural, biochemical, physiological and ecological levels of plant organization (Gniazdowska and Bogatek, 2005). The decrease in leaf chlorophyll content due to allelopathic effects has been reported (Rice,1984; Peng *et al*.,2004; Oyerinde*et al*.,2009). Apart from blocking the biosynthetic pathway of chlorophyll, allelochemicals can stimulate the degrading pathway of chlorophyll and reduce its accumulation which in turn affects photosynthesis process and diminishes the total plant growth(Rice,1984). It has been reported that the allelochemicals released to the environment by plants have significant effects on photosynthesis (Gniazdowska and Bogatek, 2005). The inhibitory effects of allelochemicals released by plants on protein content have been reported (Hamed*et al*., 2014, 2015; Hanan, 2016, Jash*et al*., 2019).
Tithoniarotundifolia(Miller) S.F. Blake is a members of the family Asteraceae. The plant associates with common crops like vegetables, cassava, yam, rice, sorghum, soyabeane.t.c. and becomes a dominant plant where it is present (Tongma*et al.*, 1998). *Glycine max* L. belongs to the family**Fabaceae and it is an** economically significant legumes grown in the tropics. It is an important oil crop grown worldwide. It is an important grain legume because of its high protein, and nitrogen fixing ability (Messina, 1997). *Sorghum bicolor*L. is an annual cereal crop belonging to the family Poacea. It is used as food for human consumption as well as food grain for animals (Moussa, 2001). This study was

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conducted to investigate the allelopathic effects of the water and methanol extracts of *Tithoniarotundifolia*. on chlorophyll and protein contents of *Glycine max* (L.) Merr. and *Sorghum bicolor* (L.) Moench

II. MATERIALS AND METHODS

A. Extraction

Extraction procedures was carried out according to the modified method of Bimal*et al*. (2016). Fresh plants of *T.rotundifolia* were harvested before flowering and separated into shoots and roots. 250 g of the fresh shoots of *T.rotundifolia* were extracted separately in water and methanol. The solution was filtered through cheese cloth to remove debris and then filtered through Whatman No 1 filter paper. The water and methanol extract solutions (100%) was diluted appropriately to give 75%, 50%, and 25% concentrations of the extracts while distilled water served as control.

B.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) and methanol extracts (100% FME,75% FME 50% FME 25% FME) daily in same quantity. Treatments were arranged in a completely randomized design (CRD) with five replications.

C. 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).

D. 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)

III. RESULTS

The accumulation of chlorophyll a in the control *G. max*plants was the highest in weeks two, three, five and six. On the other hand, the chlorophyll a content of the 100% FWE plants (68.70 – 210.38µg/g) was the lowest throughout the period of the experiment. The control plants had chlorophyll a content that was significantly different from that of the FWE plants. Significant differences were observed among the chlorophyll a content of the FWE plants at $p < 0.05$ (Fig 1a).The changes in the level of chlorophyll a in *S.bicolor* as affected by the different water extracts of *T. rotundifolia*is shown in Fig. 1b. The level of chlorophyll a in the control plants was highest from week two until the end of the experiment while that of the 100% FWE remained lowest compared to that belonging to other FWE regimes throughout the duration of the experiment.Fig. 2a & 2b show the changes in the level of chlorophyll a in *G.max and S. bicolor* as affected by the different methanolic extracts of *T. rotundifolia*. The accumulation of chlorophyll a in the control plants was higher than that of the plants in the FME regimes in the last two weeks of the experiment while that of the plants in the 100% FME regime was the lowest in most parts of the experiment. The accumulation of chlorophyll b in the control and FWE plants increased from the beginning to the end of the experiment except a

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decrease observed for plants in the 75% FWE regime. Chlorophyll b content in the control *G. max*plants was higher (48.30 – 310.69 µg/g) than that of the plants in the FWE regimes while that of the 100% FWE plant (48.3 – 180.57 µg/g) was the lowest throughout the duration the experiment (Fig. 3a).

The chlorophyll b content in the*S. bicolor* control plants was the highest from the beginning until the end of the experiment while that of the 100% FWE plants was the lowest throughout the duration of the experiment except week six (Fig 3b). The accumulation was such that the control plants had the highest chlorophyll b (212.59 µg/g) while the 100% FME plants had the lowest (103. 21 µg/g) at the end of the experiment (Fig.4a). The chlorophyll b content of the control plants was higher than that of the plants in the FME regimes in the latter weeks of the experiment while that of the 75% FME plants was lower than that of the plants in other FME regimes during the fourth and fifth weeks of the experiment (Fig 4b). Figs. 5a and 5b show the changes in the level of total chlorophyll in *G. max* and *sorghum bicolor* as affected by the different water extracts of *T. rotundifolia.* The total chlorophyll content of the control plants was the highest in most part of the experiment while that of the 100% FWE plants was lowest throughout the duration of the experiment. The total chlorophyll content of the control*G. max* plants was higher than that of the plants in the FME regimes in the latter weeks of the experiment while that of the 100% FME plants was lowest from week two until the end of the experiment (Fig.6a). The accumulation of total chlorophyll in the control*S. bicolor* plants was higher than that in the FME plants in the latter weeks of the experiment while that of the 100% FME plants was lowest in almost all the weeks except week five (fig.6b).

The protein content of the control *G. max*plants was the highest in weeks three, four five and six while that of the 100% FME plants was the lowest in most parts of the experiment (Fig.7a). The protein content of control *S. bicolor* plants was the highest in the last two weeks of the experiment while that of the 100% FWE was the lowest in almost all the weeks except the first week and last week. The control plants hadprotein content that was significantly different from that of the FWE plants. Significant differences were observed among the protein content of plants in the FWE regimes at $p < 0.05$ (Fig.7b)**.** The control*G. max* and *S. bicolor* had a protein content that was the highest while that of the 100% FME plants was the lowest in most parts of the experiment (Figs 8a and 8b)

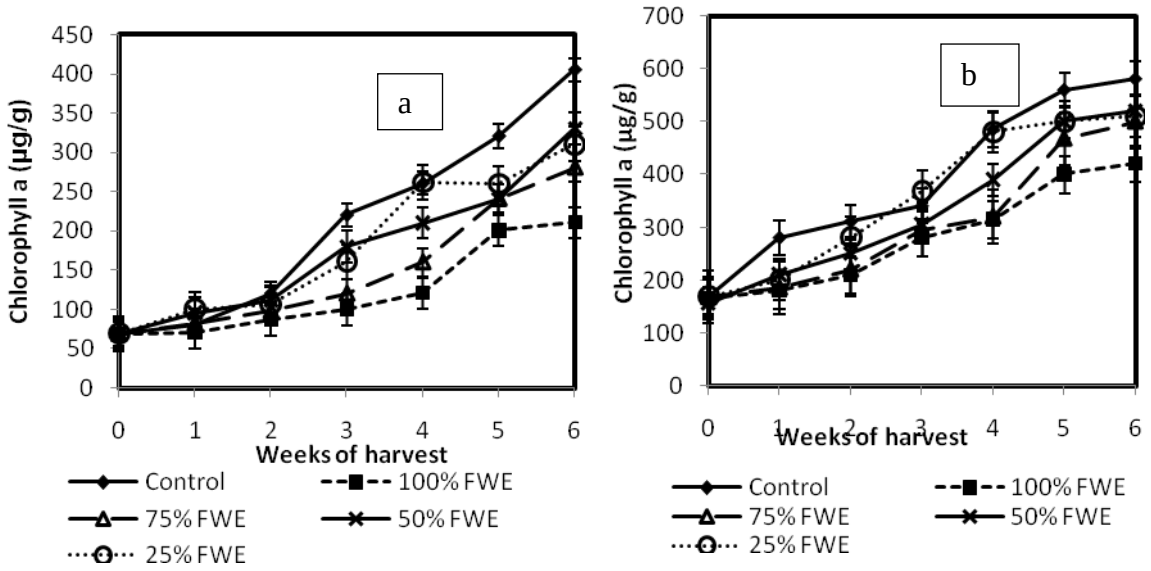

Fig.1. Changes in the level of chlorophyll a in *G.max*and S. *bicolor*as affected by the different water extracts of *T. rotundifolia*

FWE: fresh shoot water extract of *T. rotundifolia*

a. *G.max*b. *S. bicolor*

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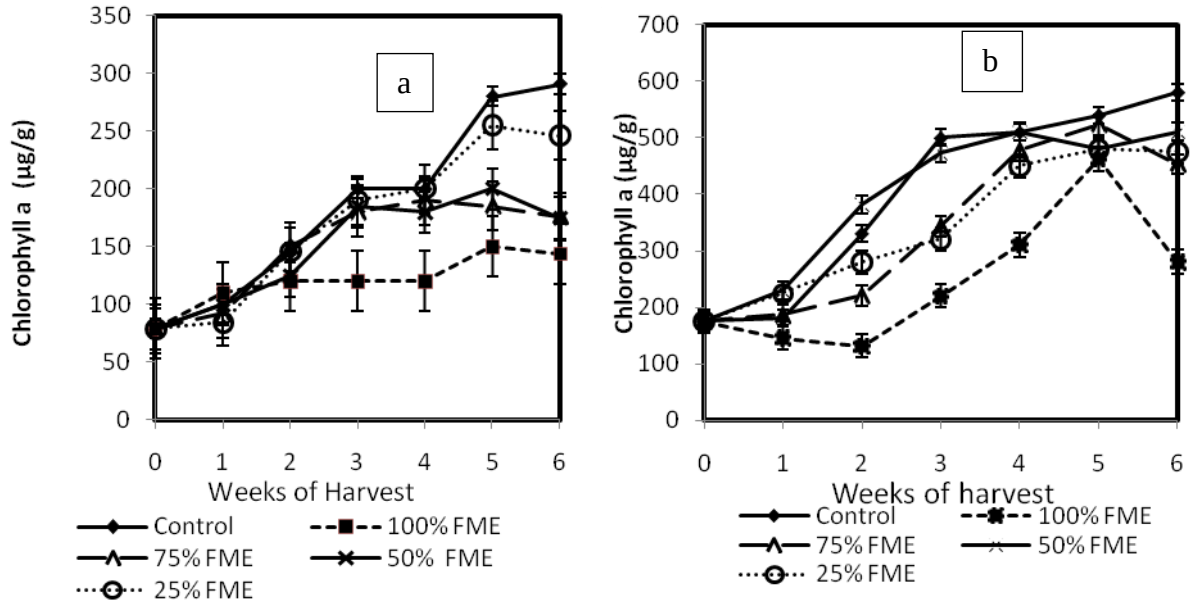

Fig. 2 Changes in the level of chlorophyll a in *G.max* and *S. bicolor* as affected by the different methanolic extracts of *T. rotundifolia*

Capped bars indicate standard errors
FME: fresh shoot methanolic extract of *T. rotundifolia*
b. *G.max*b. *S. bicolor*

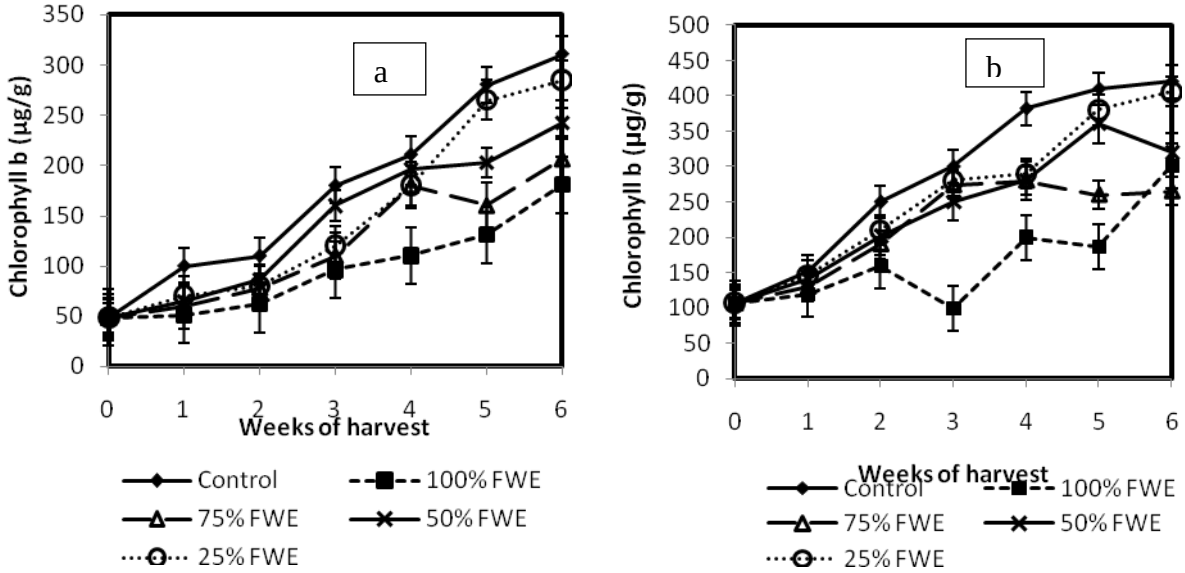

Fig. 3. Time – course of chlorophyll b formation in *G. max*and *S. bicolor*treated with water extracts of *T. rotundifolia*

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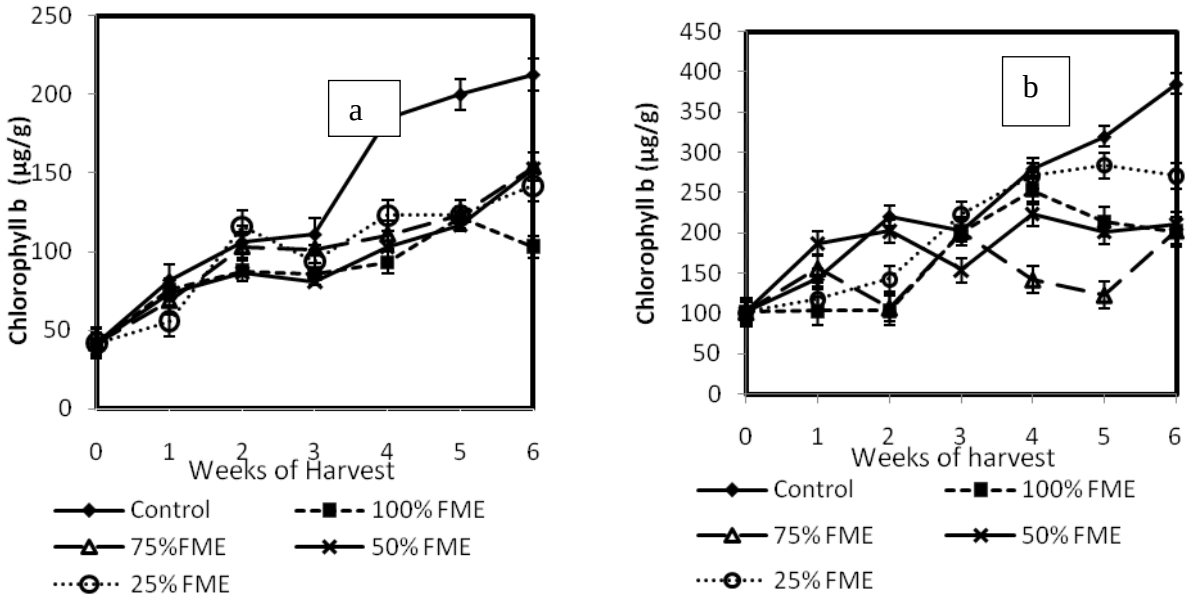

Fig.4.Time – course of chlorophyll b formation in the shoots of *G. max* and *S bicolor* treated with methanolic extracts of *T. rotundifolia*

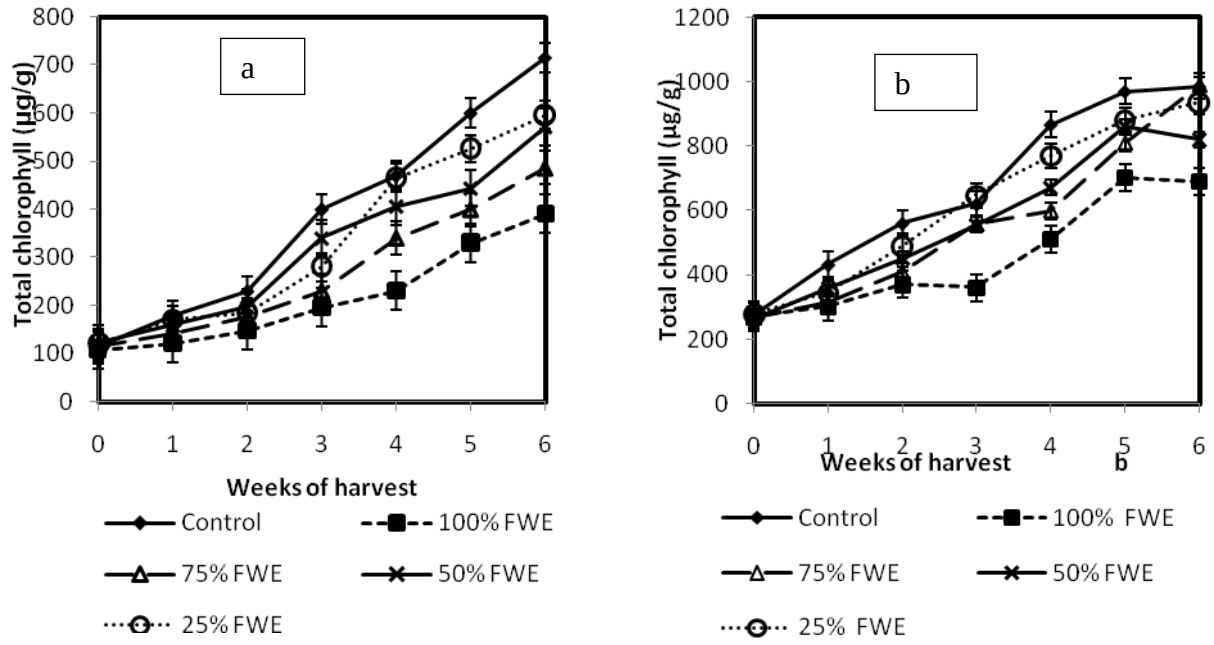

Fig. 5 Changes in the level of total chlorophyll in *G. max and S. bicolor* as affected by the different water extracts of *T. rotundifolia*

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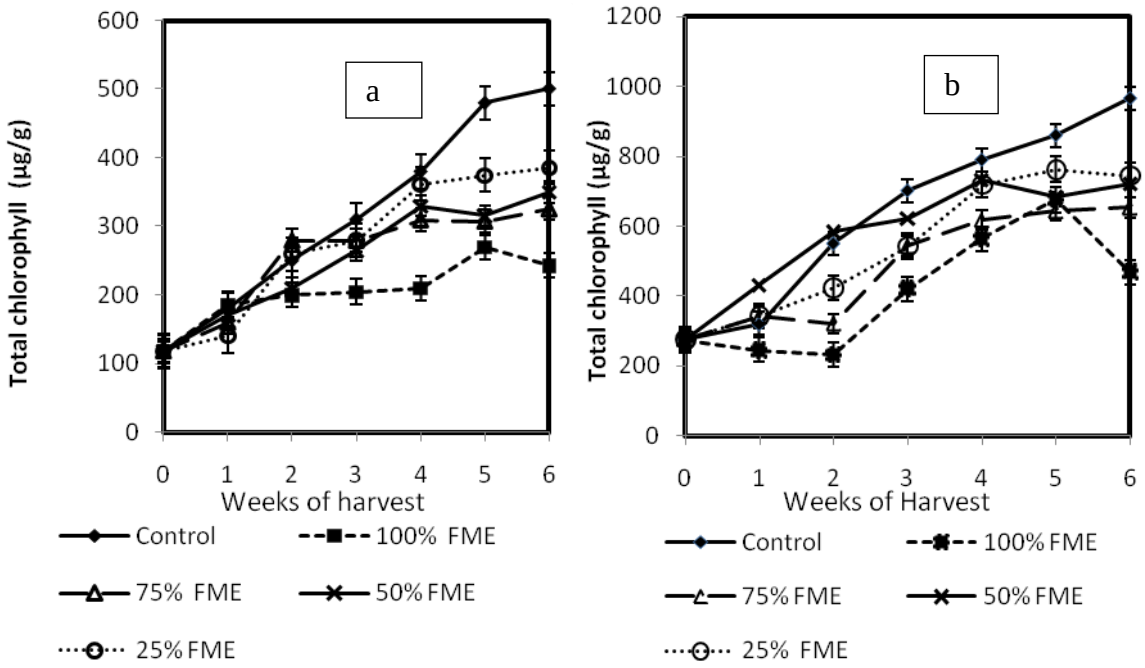

Fig. 6 Changes in the level of total chlorophyll in the shoot of *G. max* and *S. bicolor* as affected by the different methanolic extracts of *T. rotundifolia*

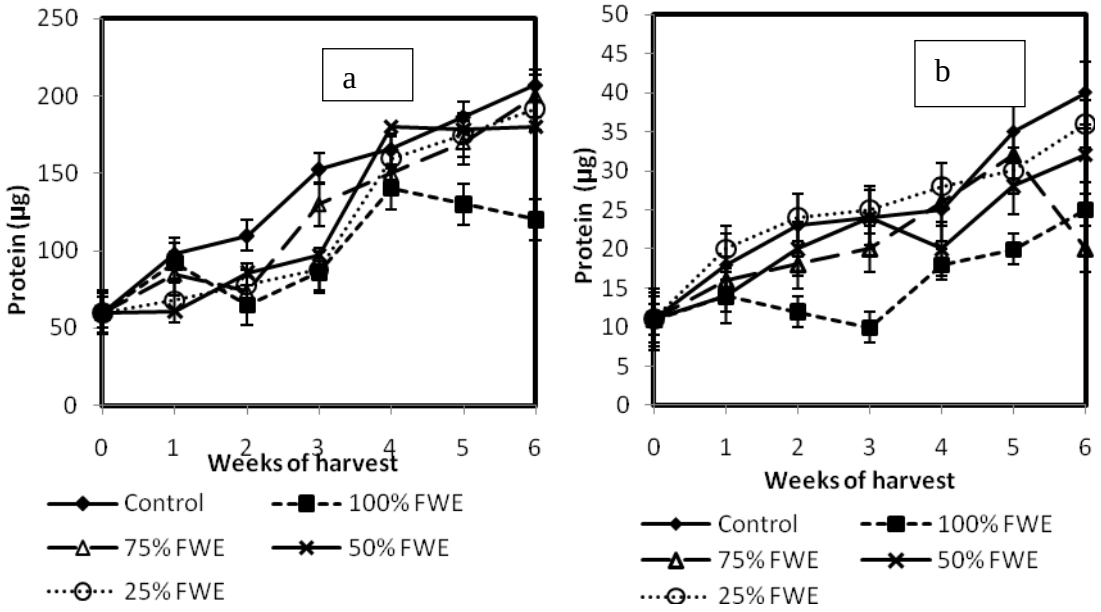

Fig.7. Changes in the level of protein in the shoot of *G. max* and *S. bicolor* as affected by the different water extracts of *T. rotundifolia*

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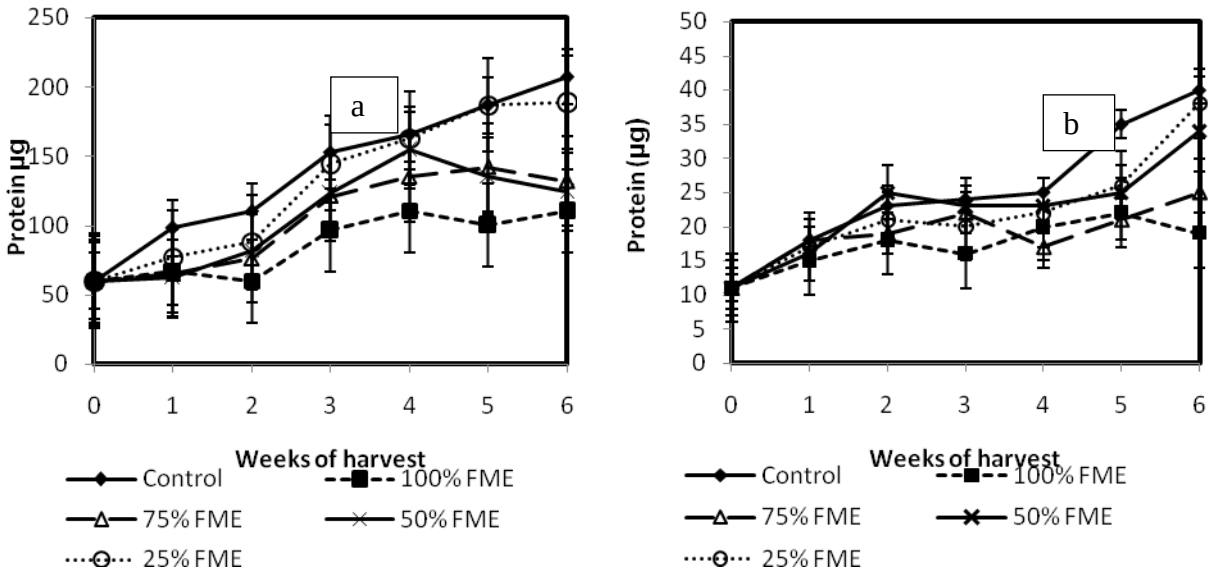

Fig. 8 Changes in the level of protein content in the shoot of *G. max and S. bicolor* as affected by the different methanolic extracts of *T. rotundifolia*

IV. DISCUSSION

Chlorophylls are photosynthetic pigments in plants, and their content and functionality are essential to absorb and direct the light to photosystems Prasad *et al.*, (2004). Therefore, a decrease in the level of chlorophyll will lead to decrease in rate of photosynthesis in plants.Chlorophyll a, chlorophyll b and total chlorophyll contents in the shoots of the test plants 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, Patterson (1981) found that allelopathic chemicals severely suppressed photosynthesis in soybean.Kapoor*et al.* (2019) observed that the aqueous leaf extracts of*Artemisia absinthium* and *Psidiumguajava*reduced the level of chlorophylls and carotenoids, which suggests possible photosynthetic limitations exerted by both the extracts to *Parthenium*leaves.According to Maura *et al.* (2002), the alteration of photosynthesis by allelochemicals could be as a result of the disruption of election transport chain and alleration in chlorophyll biosynthesis. Yang *et al.* (2002) stated 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. According to Siddiqui and Zaman (2005), apart from blocking the biosynthetic pathway of chlorophyll, allelochemicals can stimulate the degrading pathway of chlorophyll and reduce its accumulation which in turn affects photosynthesis process and diminishes the total plant growth. Allelochemicals can reduce the chlorophyll and porphyrin content and in turn affecting photosynthesis and the total plant growth (Siddiqui and Zaman, 2005). The photosynthesis potential in plants is directly proportional with the chlorophyll content present in leaf tissues which play an important role in photochemical reactions (Schlemmer*et al.*, 2005). According to Inderjit and Duke (2003), allelochemicals can inhibit PSII components and ATP synthesis.

In the initial weeks of the experiment, the total protein of *S. bicolor* plants treated with the 25% water extract were significantly stimulated compared to the control plants. This stimulation correlated with stimulation in nucleic acid content. As p-coumaric acid increased incorporation of 3S S methionine into protein (Baziramakenga*et al.*,

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1997).Sustained application of the extracts over the weeks led to the accumulation of the allelochemicals in the soil which consequently resulted in the inhibition of the total protein accumulation in all the test crops in the latter weeks of the experiment. This observation was supported by the report of Hall *et al.* (1989) that allelochemicals have to accumulate in sufficiently high quantity in the soil to be phytotoxic enough to cause inhibitory effects.Mersia and Singh (1993) reported a reduction in protein synthesis due to the use of synthetic allelochemicals in treating scalet rose and leaf cells of velvet leaf (*Abutitiontheophrasti*). The inhibition of protein content was due to the presence of allelochemicals in shoot extracts of *T. rotundifolia*. According to Hamed and Ahmed (2014), reduction in total soluble protein by allelochemicals may be attributed to the effect of these allelochemicals on DNA replication or transformation by intercalation with nucleic acids by ionic bonding with their negatively charged phosphate groups. Also, it is possible that allelochemicals in extracts of *T. rotundifolia* may reduce the incorporation of certain amino acids into proteins and thus reduced the rate of protein synthesis. Cinnamic acid derivatives depressed translation activity of polysomalmRNAase of bean cells which reduced protein synthesis (Bolwell*et al*., 1988). According to Hegab and Ghareib, (2010), accumulation of phenolic glycine interferes with the cytoplasmic ribosomes and production of RNA, which in turn inhibited protein synthesis. The observed retardation of protein accumulation by the extracts could be the result of interference in the protein biosynthetic pathway. Alternatively, it could have been caused by the inhibition or alteration of the action of some relevant enzymes or by the allelochemicals complexing with the synthesized protein.

V. CONCLUSION

T. rotundifolia had allelopathic potential which reduced chlorophyll and protein contents of the test crops. This study showed that allelochemicals in *T.rotundifolia*were soluble and better extracted by the organic methanolic solvent than water as the methanolic extract being more phytotoxic than the water extracts had a more pronounced retardatory effects on the chlorophyll and protein contents of the test crops.

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