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SUSCEPTIBILITY OF *Glycine max* (L.) MERR.TO THE CRUDE WATER EXTRACT FROM THE LEAVES OF *Tithonia diversifolia* (HEMSL.) A. GRAY UNDER NURSERY CONDITIONS

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Abstract

The growth and performance of *Glycine max* (L.) Merr. (variety T.G.X.1448-2E) under nursery conditions were studied using the crude water extract from leaves of *Tithonia diversifolia* (Hemsl.) A. Gray as treatments at varying concentrations. The phytotoxic effects of the extracts from leaves of *T. diversifolia* were studied on seed germination, plant height, dry weight (biomass) and yield of *Glycine max.* The field experiment was carried out at LAT. N07°26'35.6"; LONG.E003°53'46.3"and elevation 205m to 207m. Twenty experimental hips of height 0.4 metres each were constructed separately one metre apart from one another in rectangular piece of land of 9m x 8m cleared plot and laid out in a Completely Randomized Block Design (CRBD) with total of five treatments in quadruple for the treatments namely: 100% w/v crude extract (T_1), 50% w/v crude extract (T_2), 25%w/v crude extract (T_3), 12.5%w/v crude extract (T_4) and Control (T_5) which was 6 litres equivalent of water. The degree of the susceptibility of *G.max* to the phytotoxicity was significant. Significant retarded growth was obtained for all the treatments T_1, T_2, T_3 and T_4 at 95% confidence interval. 100%w/v crude extract (T_1) showed the highest level of significance on stem height and girth ($P \leq 0.05$). Biomass and yield showed no significant effect on any of these treatments. This studies revealed that the phytotoxins in leaves of *T. diversifolia* was inhibitory to the performance and growth of this economic crop.

Keywords: Phytotoxicity, Susceptibility, Biomass, inhibitory.

Introduction

Glycine is a genus in the bean family Fabaceae formerly referred to as Leguminosae. The best-known species is *Glycine max* (L.) Merr. commonly known as soybean. *G. max* (L.) Merr., is a diploidized tetraploid (2n=40), the subfamily Papilionoideae, the tribe Phaseoleae, the genus Glycine Wild and the subgenus Soja (Moench) (Ratnarparkhe, *et al.*, 2011; OECD, 2012,). It is an annual, erect and bushy herbaceous plant that can reach a height of 2metres. Satpute and Pathare (2018) established that three types of growth habits can be found amongst soybean cultivars, namely; determinate, semi-determinate and indeterminate. Determinate growth is characterized by the cessation of vegetative

activity of the terminal bud when it becomes an inflorescence at both axillary and terminal racemes. Determinate genotypes are primarily grown in the southern USA. Indeterminate genotypes continue vegetative activity throughout the flowering period and are grown primarily in central and northern regions of North America. Semi-determinate types have indeterminate stems that terminate vegetative growth abruptly after the flowering period. None of the soybean varieties are frost tolerant, and they do not survive Canadian winter conditions (Lu, 2004). *G. max* belongs to the subgenus Soja which also contains *G. soja* and *G. gracilis*. *G. soja*, a wild species of soybean, grows in fields, hedgerows, roadsides and riverbanks in many Asian countries. Cytological, morphological and molecular evidence suggest that *G. soja* is the ancestor of *G. max. G. gracilis* is considered to be a weedy or semi-wild form of *G. max*, with some phenotypic characteristics intermediate to those of *G. max* and *G. soja. G. gracilis* may be an intermediate in the speciation of *G. max* from *G. soja* or a hybrid between *G. soja* and *G. max* (OECD, 2000; Lu, 2004 and CFIA, 2012).

Glycine max is a leguminous species grown for its edible bean with numerous uses. The plant is classed as an oil seed rather than a pulse (FAO, 2003). It is an important source of high quality but inexpensive protein and cooking oil. Wilcox (1987) reviewed that it contains 41% proteins and 21% oil. It consists of more than 36% protein, 30% carbohydrate and excellent amount of dietary fibres, vitamins, minerals as well as 20% oil which make it the most important crops for producing edible oil (IITA, 2014). The significance of soybean oil in the production of biodiesel has led to increment industrial use of soybean oil in Countries like Argentina, Brazil and the US by 187%, 208% and 101% respectively over the year 2007-2008 (Dwevedi *et al.*, 2011). However, Argentina still dominate world soybean oil exports satisfying about 72% of the world market while Brazil and the US together account for 21% of the world soybean oil net export by 2017 to 2018 despite the domestic biodiesel mandate. China and Indian combined share the net imports hold 48% of the world market. In sub-sahara Africa, Nigeria is the largest producer of soybean followed by South Africa (IITA, 2014). It enjoys enhancing cultivation in the rainforest agro-ecological zone as well as savannah because it is a major cash crop widely used in food and feeds (Okpara and Ibiam, 2000; Sangiga *et al.*, 2002; Ofor and Okpara, 2005 and Okpara *et al.*, 2007). Shortage of fertilizer constraints the ability of some countries to increase production. Although development and introduction of new varieties of cultivated soybean have led to an increase in the production of this important crop in Nigeria, sustainable production is constrained by poor soil fertility (Ogoke *et al.*, 2003). Dependence on biological means of maintaining soil fertility is necessitated by high procurement of cost of chemical. Reduced soil acidity owing to low levels of calcium (Ca), magnesium (Mg), phosphorus(P), potassium (K), and micronutrients such as boron (B), zinc (Zn) and low pH, increase or high level of aluminium (Al) and manganese (Mn) lowers soybean yield (Fageria, 1994). Soil fallowed with *Tithonia diversifolia* showed significantly higher organic matter in N, P, K, Ca, and Mg (Taiwo and Lawan, 2014). Investigation showed that *T. diversifolia* green manure and *Eichhornia crassipes* (Mart) Solms (water hyacinth) on soil have high fertilizing potential; with sound potential for building soil organic matter to adequate levels that will build nutritional needs of crops as well as

improve the nutrient elements of state of nutrient depleted soils into which such organic resources are incorporated (Chukwuka *et al.*, 2009) and been used to increase the yield of crops (Olabode *et al.*, 2007; Ilori *et al.*, 2010; Ademiluyi, 2012). Conversely, data on the detrimental effects of weeds particularly those belonging to the Asteraceae family on some plant species abound (Eze and Gill 1992; Onwugbuta 2001; Chon *et al.*, 2003; Maharjan *et al.*,2007; Ilori *et al.*, 2010; Abu-Romman 2011; Al-watban and Salama 2012). They are characterized as predominating plantations of useful cash crops. *Tithonia diversifolia* is a common invasive weed in Africa and Nigeria in particular (Akobundu and Agyakwa, 1998). The leave extract of *Tithonia diversifolia* has been observed to have inhibitory effects on some plants growth (Chukwuka *et al.,* 2013). Be it as it may, it could not be vividly concluded that phyto–growth inhibitor, allele-chemics or allelochemicals or phytotoxins are responsible for such a phyto-growth behaviour. Many of the phytotoxins are water soluble substances released into the environment through leaching, root exudation, volatilization and decomposition of plants residues and are affected by many environmental factors (Reigosa *et al.,* 1999). The chemical compounds interfere with the growth of other plants and often adversely affect the yield of other plants through the process of allelopathy (Ferguson *et al.,* 2003). A number of biologically active compounds, predominantly the secondary metabolites, has been isolated from different parts of Mexican sunflower (Schuster *et al.,*1992; Chan *et al.*,2000; Taiwo and Makinde, 2005; Otusanya and Ilori, 2012). Stiles *et al.* (1994) established the fact that some of the secondary metabolites are well known as plant growth inhibitors. It has thus been averred that this plant possesses a lot of potentials in terms of growth- inhibiting as well as growth stimulating properties vis-à-vis several other plants, especially, crops (Chukwuka *et al.*, 2013).

This experimental research was, ipso-facto carried out to determine the susceptibility of *Glycine max* to phytotoxic activity of leaves crude water extract from *Tithonia diversifolia* and its effects on dry weight (Biomass) and yield of the *G. max* under nursery conditions.

Materials and Methods

The study was carried out in the screen house of the Department of Botany, University of Ibadan, Oyo State, Nigeria. The area lies between Latitude 3.53^{0}E and 7.26^{0}N and altitude of 185m above sea level (Akin-oriola, 2003; Chukwuka and Uka, 2007) with a mean daily temperature of 24.6^{0}C. A rectangular piece of land of 9m x8m was cleared and used for the study. Twenty-five hips (top soil) of height0.4m each were constructed separately one meter apart from one another and layed in Completely Randomized Block Design(CRBD) with five treatments and five replications namely: 100% w/v crude leaf extract of *Tithonia diversifolia* (T_1), 50% w/v crude leaf extract of *Tithonia diversifolia* (T_2), 25% w/v crude leaf extract of *Tithonia diversifolia* (T_3), 12.5% w/v crude leaf extract of *Tithonia diversifolia* (T_4) and Control (T_5) which did not receive leaf extract from *Tithonia diversifolia* other than 6litresequivalent amount of water. Six kilogram each of the plant materials was washed thoroughly with tap water, cut into fine pieces and finely grounded using a ceramic pestle and mortar, the ground plant materials was allowed to stand into two separate bowls for 12 hours with 6 litres of distilled water added to each bowl. The solute was separately sieved to obtain 12 litres of crude extracts from each bowl as 100% w/v aqueous extract of *T. diversifolia* (T_1). Solution of lower concentrations was

prepared from the crude extract. A solution of 50% w/v, 25% w/v and 12.5% w/v which were taken as T_2, T_3 and T_4 respectively for *Tithonia diversifolia* were prepared by adding equivalent or proportional volume of distilled water to the crude extract until the desired concentrations of the extracts were obtained. That is, equivalent volume of distilled water was added to 100% w/v crude extracts to obtain 50% w/v concentration designated as T_2. This was repeated for 50% w/v concentration to obtain25%w/v concentration that was tagged T_3 which was in turn added to its equal volume of distilled water to obtain 12.5% w/v concentration of *Tithonia diversifolia*. This made up a total of four treatments from *T. diversifolia* (i.e., T_1, T_2, T_3 and T_4) at 100% w/v, 50% w/v, 25%w/v and 12.5% w/v respectively and T_5 was as well prepared to serve as the control which contained 6 litres equivalent volume of distilled water only for each of the treatment that was used.

Eight seeds of *G. max* variety T.G.X. 1448-2E obtained from International Institute of Tropical Agriculture (IITA), Ibadan,with their seed coats uncracked which signified undamaged endosperm were sorted, washed with tap water and planted in each of the experimental hips on the field and was regularly watered alternate days interval with about six litres of volume of water to enhance smooth germination and prevent wilting due to insufficient or shortage of water until the supplied with the different concentration of the treatments at 4 weeks after sowing (4WAS).

Table 1: Physico-chemical properties of the soil before treatment were applied.

Property	Unit	Value
Sand	g/kg	872.00
Silt	g/kg	114.00
Clay	g/kg	14.00
pH in H2O	-	5.70
Total nitrogen	g/kg	1.51
Total organic carbon	g/kg	14.42
Exchangeable acidity H+	cmol/kg	0.70
Exchangeable acidity Al3+	cmol/kg	-
Calcium	cmol/kg	5.54
Magnesium	cmol/kg	0.90
Sodium	cmol/kg	0.69
Potassium	cmol/kg	0.36
Zinc	mg/kg	12.68
Copper	mg/kg	2.34
Iron	mg/kg	91.20
Manganese	mg/kg	133.00

The experimental soil samples were analyzed for nutrient status prior to their experiment for their physical and chemical properties. The results of the soil analysis are shown in Table 1. At Four weeks after sowing (4WAS), the experimental hips were thinned down to one seedling per hip and treatment. The performance and growth parameters such as stem girth- diameter of the shoot which was measured with an electronic device called Mitutoyodigitamatic

electronic caliper (MDEC) Model Cd- 8" p; produced by Mitutoyo America Corporation, America; leaf length- the distance between the apex and the leaf base; leaf width- the distance between the leaf blades in the widest part of the leaf; and plant height as the height from the base of the plant to tip of the plant were measured with meter rule and recorded. This was done subsequently on weekly basis. First day and fifty percent day of flowering as well as the day fruiting started and fifty percent days of fruiting were noted as the number of the days from sowing to when flowering started and half of the plant sown in the plot has fruited respectively. The soybeans was harvested at three months after planting (3MAP) when 85-90% of the pods had turned brown and it was believed to have attained physiological maturity by pulling the whole dry plant with the roots. At this age fourteen weeks after sowing (14WAS), soybeans stands were carefully uprooted with the aid of a spade to maximize any root damage to the barest level. The roots were dipped in 30 litres of tap water filled in a bowl and the attached soils were carefully washed off. They were then washed repeatedly under running tap water until there was no trace of soil and subsequently rinsed again with distilled water. Harvested stands and soybean pods were sun-dried and later oven-dried at a temperature of 80^{0}C for 48 hours to remove all available moisture content present after which it was allowed to cool inside the oven. Dry weight of seed (yield), pods and leaves + shoot + root per plant (Biomass) were measured with the aid of Labman digital electronic weighing balance model MAB 220, produced by Swastik System and Services, Shahdara, New Delhi, India,and leaf area of trifoliolate was calculated using the equation of Wiersm and Barley (1975):

$$A = 0.411 + 2.008\ L\ W$$ (Terminal leaflet)

Where A = trifoliate leaf area;

L = maximum length;

W = width of the terminal leaflet of a trifoliate leaf;

and 0.411 and 2.008 were constant.

All the data obtained were subjected to statistical analysis using one way analysis of variance with statistical package for social sciences (SPSS v. 16) version 16 software (SPSS Inc. Chicago, IL, USA), and mean separation was done using Duncan's Multiple Range Test (DMRT)($P \leq 0.05$).

Results and Discussion

Table 2 shows the effects of each of the treatments applied on the height of *Glycine max* from four weeks after sowing (4 WAS) to fourteen weeks after sowing (14WAS). The plant height increases progressively with increase in week from week 4 after sowing to 14 WAS. Generally, the fastest growth is observed between week 5 and week 6 after sowing among all treatments. The plants rapidly and progressively grew and developed from week 4 after sowing to week 7 after sowing. At this stage, the plantlets were said to be passing through a logarithmic phase of growth after which their growth seemed to be progressively stable which could be interpreted as a stationary phase of development leading to the senescence of the plants. At weeks between the logarithmic phase of the growth behavior of the plants could be attributed to the softness of the plant tissues. The plant tissues were still young and meristematic cell division and active proliferations of the plant cells were still invigorating. The tenderness of the plant

made them able to acquire much essential requirements for growth such sufficient water as much as desired from the soil which support their rapid growth and development. This phase prepared the plant for the flowering and fruiting phase in the control (T_5) and among the plants treated with the leave extracts. Flowering was observed in the day 2 of the 6th week after sowing (6WAS) tantamount to 41 days after sowing (41 DAS) for all (T_1, T_2, T_3, T_4 and T_5) and first day of the 7th week after sowing for group of *Glycine max* which were treated with water. This make up 47days after sowing (47 DAS). Fifty percent (50%) days of flowering as the number of days from sowing to when half of the *Glycine max* variety T. G. X. 1448–2E sown in the plot has flowered was observed to be forty-five days after sowing (45DAS). Which appeared the fourth day after the first day flowering was observed. This implies that control was among the plants that blooms late. First day of fruiting which is defined as the day from sowing (0DAS) to the days fruiting was observed was fifty- three- day (53-day) after sowing equivalent to 8 weeks after sowing (8 WAS). Fifty percent day of fruiting (50%) was observed to be in the second day of 9week after sowing (9 WAS). This make up 62-day after sowing (62-DAS).

At this period a drastic reduction in the rate of growth and development was observed. Starting from the 6-week after sowing (6WAS), which marked the beginning of flowering, there were slightly reduced but considerable progressive increase in height to 7 weeks after sowing (7WAS) which marked the beginning of fruiting and growth almost completely stabilized and even stopped between 8, 9, 10, 11, 12, 13 and 14-week after sowing (8WAS). Most of the energy used for growth and development has been channeled into flowering and fruiting. This consequently reflected in the laggard growth observed during the flowering and fruiting phase of the plant. The phase for flowering, fruiting and maturity of the fruit for the group of *Glycine max* which were treated with leaf extract took longer duration than that of the control which produces few flowers and pods whereas more flowers were increasingly produced and developed into pods. At 13 and 14 Week after sowing there were no observable increase in height and 85-95% *Glycine max* leaves and pods changed colour from green to brown and it was believed to have attained the physiological maturity – state at which all physiological activities ceased.On weekly basis, it was observed that there were no significant effects of the treatment($P \leq 0.05$)on the plant height among treatments on week 4 and 6 after sowing, whereas, reverse was the case in other weeks in which the effects of the treatments on *Glycine max* among the treatments in week 6, 7, 8, 9, 10, 12, 13 and 14 showed significant different from one another. Though the height of the *Glycine max* varied among treatments T_1, T_2, T_3, T_4 and T_5 for 4 and 5 weeks after sowing (WAS), they were statistically not different from one another. At this stage, all the plants were young and they were still not responsive to the effect of the treatments. These growth and development approximately almost at the same rate, whereas, the response of the plants to treatments among week 6, 7, 8, 9, 10, 11, 12, 13 and 14 after sowing differs. This resulted on significant differences in heights observed among the plants. In 6 weeks, T_5(25.63$\pm$1.39cm) was significantly different from all the treatments. Treatment T_2(122.77$\pm$3.80cm), T_3(21.13$\pm$2.65cm), T_4(19.90 $\pm$ 2.96cm) showedno significant difference in height of Glycine max as well as treatment T_1(15.73$\pm$2.28cm). T_1(15.73$\pm$2.28cm) is significantly lower in height to the control (T_5). At 7WAS, the effect of the treatment ($P \leq 0.05$) was significantly

higher in the control (T_5) (26.37±1.84cm) among all the treatments. These pattern of least significant effects of crude extract of *Tithonia diversifolia* (T_1) on the growth of*Glycine max* and highest significant effect of the control (T_5) was observed among all the treatments for 7, 8, 9, 10, 11, 12, 13 and 14 weeks after sowing (WAS). This implies that*G. max* treated with crude extracts(T_1) exhibited significant susceptibility to the phytotoxins in the leaf extract which was evident in the inhibitory growth effects on the plant. This is in congruence with the finding of Tongma *et al.* (1998) whose observation revealed that *Tithonia diversifolia* contained some allelochemicals and therefore suggested as being capable of passing a serious threat of phytotoxicity to agricultural crops. Also, as the concentration of *Tithonia diversifolia* decreases (i.e. on dilution), the effects of the treatment becomes prominently significant except T_3 (25%w/vcrude extracts of *Tithonia diversifolia)* with 25.30 ± 3.82cm, 26.07 ± 3.78cm, 26.33 ± 3.92cm, 26.43 ± 3.77cm, 26.83 ± 3.9cm, 26.97 ± 4.03cm, 27.13 ± 4.12cm and 27.13 ± 4.12cm for 7, 8, 9, 10, 11, 12, 13 and 14 weeks after sowing (WAS) that were less diluted and observed more significantly higher than T_4 (12.5%w/v crude extract of *Tithonia diversifolia*) with 21.40 ± 2.36cm, 22.07 ± 2.99cm, 22.50 ± 3.01cm, 22.57 ± 3.09cm, 22.83 ± 3.23cm, 22.83 ± 3.23cm, 22.90 ± 3.21cm and 22.90 ± 3.21cm for 7, 8, 9, 10, 11, 12, 13, and 14 weeks after sowing (WAS) as shown in table 2. *Glycine max* with the highest significant growth was observed with T_3 while the least significant growth was observed for T_1. Some weeds in the family asteraceae was found a reservoir of allelochemicals or allelochemics or phytotoxins especially in leaves (Eze and Gill,1992) which inhibit the growth of many plants (Tet-vun, 2002, Onweighta-Enyi, 2001; Ilori et al., 2009). The phytotoxic inhibition exhibited by *Tithonia diversifolia* has been attributed to the sesquiterpene lactones (Franscisco *et al.*, 2006, Maria *et al.*, 2015).

Table 2: Effect of treatment on plant height (cm)

TREATMENT	4WAS	5 WAS	6 WAS	7 WAS	8 WAS	9 WAS
T_1	12.03±2.25	13.73±2.42 a	15.73±2.28	16.37±2.29	16.87±2.28 c	17.17±2.26
T_2	8.90±3.10	12.83±3.18 a	22.77±3.80 b	23.77±2.70 bc	24.07±2.56 abc	24.17±2.69 bc
T_3	9.77±0.72	14.90±1.15 a	21.13±2.65 b	25.30±3.82 b	26.07±3.78 ab	26.33±3.92 b
T_4	11.50±0.45	16.17±2.09 a	19.90±2.96 b	21.40±2.36 bc	22.07±2.99 abc	22.50±3.01 bc
T_5	10.90±0.36	18.07±0.26 a	25.63±1.39	26.37±1.84	27.50±2.40 a	29.67±2.67
TREATMENT	**10 WAS**	**11 WAS**	**12 WAS**	**13 WAS**	**14 WAS**	
T_1	17.50±2.19	17.70±2.21 c	17.87±2.12	17.97±2.10	17.93±2.07 c	
T_2	24.33±2.78 b c	24.40±2.81 abc	24.43±2.78 c	25.13±3.23 bc	24.47±2.80 abc	
T_3	26.43±3.77 ab	26.83±3.97 ab	26.97±4.03 ab	27.13±4.12 b	27.13±4.12 ab	
T_4	22.57±3.09 c	22.83±3.23 abc	22.83±3.23 c	22.90±3.21 bc	22.90±3.21 abc	
T_5	29.80±2.70	30.37±2.62 a	30.37±2.62a	30.43±2.68	30.43±2.69 a	

T_1 = 100% w/v crude extract of *Tithonia diversifolia*; T_2 = 50% w/v extract of *Tithonia diversifolia*;
T_3 = 25% w/v extract of *Tithonia diversifolia*; T_4 = 12.5% w/v extract of *Tithonia diversifolia*; T_5 = Control: equivalent amount of water; WAS = Weeks after sowing. Mean ± SEM (n=5). The columns with the same letter are not significantly different at P≤ 0.05 using Duncan's multiple range test.

Table 3 showed the effects of each of the treatments applied on the stem girth of *Glycine max* from 4 weeks after sowing to 14 weeks after sowing (14 WAS). At 4WAS, the effects of the treatments on the plant girth of *G. max* were not significantly different among treatments expect for T_4 (0.18±0.01cm). T_4>T_1, T_2, T_3 and T_5.

Table 3: Effect of the treatments on stem girth (cm)

TREATMENTS	4WAS	5 WAS	6 WAS	7 WAS	8 WAS	9 WAS
T_1	0.15±0.02^{ab}	0.17±0.02^{ab}	0.20±0.02^{ab}	0.20±0.02ab	0.21±0.02b	0.22±0.02ab
T_2	0.15±0.01^{ab}	0.19±0.01^{ab}	0.22±0.01^{ab}	0.24±0.02ab	0.27±0.04ab	0.28±0.04a
T_3	0.13±0.02^{ab}	0.19±0.01^{ab}	0.22±0.02^{ab}	0.25±0.01ab	0.27±0.00ab	0.29±0.02a
T_4	0.18±0.01^{a}	0.20±0.01^{ab}	0.24±0.01^{a}	0.26±0.00a	0.30±0.03a	0.26±0.03ab
T_5	0.15±0.01^{ab}	0.19±0.00^{ab}	0.24±0.01^{a}	0.25±0.01ab	0.27±0.02ab	0.29±0.01a
TREATMENTS	10 WAS	11 WAS	12 WAS	13 WAS	14 WAS	
T_1	0.21±0.02b	0.20±0.02b	0.19±0.02b	0.19±0.02b	0.19±0.02b	
T_2	0.29±0.05ab	0.29±0.05ab	0.28±0.05ab	0.28±0.06ab	0.28±0.06ab	
T_3	0.30±0.03ab	0.30±0.03ab	0.30±0.03a	0.35±0.04a	0.35±0.04a	
T_4	0.28±0.01ab	0.28±0.01ab	0.28±0.01ab	0.28±0.01ab	0.28±0.01ab	
T_5	0.28±0.01ab	0.30±0.04a	0.29±0.04ab	0.29±0.04ab	0.29±0.04ab	

T_1 = 100% w/v crude extract of *Tithonia diversifolia*; T_2 = 50% w/v extract of *Tithonia diversifolia*; T_3 = 25% w/v extract of *Tithonia diversifolia*; T_4 = 12.5% w/v extract of *Tithonia diversifolia*; T_5 = Control: equivalent amount of water; WAS = Weeks after sowing. Mean ± SEM (n=5). The columns with the same letter are not significantly different at P≤0.05 using Duncan's multiple range.

Table 4 showed the effects of the treatments on the terminal leaf length of *Glycine max* from 4 to 14 weeks. Generally, there was an increase in leaf length with plant age (as week progresses) in all the treatment groups. On weekly basis, there were significant differences of the mean values (P ≤0.05) among the treatments from one week to the other. At 5WAS, T_5(4.47±0.58cm) was highly significant (P ≤ 0.05) than T_2(5.93±0.65), T_3 (5.83±0.15cm), T_4(5.90±0.46cm)> T_1 (4.87±0.58cm) and this trend was repeated in 6WAS. The mean values of the control(T_5) were observed to be the most significant among the treatments in 6, 7, 8, 9, 10, 11, 12, 13 and 14 WAS. At 11WAS, T_5 (7.67± 0.87cm) was significantly higher in leaf length than the groups of soybean treated with crude extract of *T. diversifolia* (T_1 = 6.43±0.47cm) while T_3 (8.33±0.19) showed no significant different from each other but were significantly higher than T_4(7.23±0.68).

Table 4: Effects of the treatments on terminal leave length (cm) of *G. max*

TREATMEN	4WAS	5 WAS	6 WAS	7 WAS	8 WAS	9 WAS
T_1	4.13±0.58l	4.87±0.58bc	5.47±0.66	5.70±0.64a	5.90±0.61	5.90±0.62
T_2	5.23±0.62a	5.93±0.65ab	6.47±0.61a	7.53±1.34	7.67±1.3	7.87±1.27
T_3	5.17±0.17a	5.83±0.15ab	7.43±0.32	7.70±0.42a	7.83±0.3	7.93±0.28
T_4	5.20±0.35a	5.90±0.46ab	6.23±0.57a	6.50±0.62a	6.70±0.61	6.87±0.64a
T_5	6.80±0.59	7.63±0.64a	8.00±0.76	8.10±0.71	8.30±0.6	8.40±0.65
TREATMEN'	10 WAS	11 WAS	12 WAS	13 WAS	14 WAS	
T_1	6.27±0.50b	6.43±0.47bc	6.57±0.41	6.80±0.32c	6.80±0.32	
T_2	8.17±1.16a	8.37±1.11ab	8.63±1.00	8.73±0.95a	8.73±0.95	
T_3	8.10±0.20a	8.33±0.19ab	8.47±0.17	8.63±0.13a	8.63±0.13	
T_4	7.03±0.68a	7.23±0.68ab	7.50±0.56a	7.73±0.47l	7.73±0.47	
T_5	8.53±0.68	8.93±0.58a	9.33±0.48	9.60±0.35	9.67±0.3	

T_1 = 100% w/v crude extract of *Tithonia diversifolia*; T_2 = 50% w/v extract of *Tithonia diversifolia*; T_3 = 25% w/v extract of *Tithonia diversifolia*; T_4 = 12.5% w/v extract of *Tithonia diversifolia*; T_5 = Control: equivalent amount of water; WAS = Weeks after sowing. Mean ± SEM (n=5). The columns with the same letter are not significantly different at P≤ 0.05 using Duncan's multiple range test.

Table 5 showed the effects of the treatment on the terminal leaf breadth of *Glycine max.* The mean values of the leaf breadth of *Glycine max*increases progressively with increase in age of the plants from 4 to 14 weeks after sowing. The mean values were significantly different ($P \leq 0.05$) among treatment T_1, T_2, T_3, T_4 and T_5. Generally, the control (T_5) was observed to have significantly wider mean values among all the treatment from 4 WAS to 14 WAS. The group of the plants treated with equivalent amount of water had wider leaf than the other groups. At 4 WAS, T_5 (4.37±0.67) was significantly higher than $T_1$and T_4 whose effects on the width of *G.max* were not relatively significant but were more comparatively significant ($P \leq 0.05$) to T_2 and T_3 that in turns had no significant difference from one another.

Table 5: Effects of the treatment on the terminal leaf breadth of *Glycine max*

TREATMENTS	4WAS	5 WAS	6 WAS	7 WAS	8 WAS	9 WAS
T_1	2.57±0.54b	2.73±0.55b	3.13±0.64b	3.30±0.60b	3.40±0.61b	3.50±0.59b
T_2	3.17±0.38ab	3.40±0.42ab	3.60±0.40ab	3.83±0.38ab	3.93±0.38ab	4.03±0.38ab
T_3	3.60±0.06ab	3.80±0.06ab	4.03±0.12ab	4.20±0.12ab	4.23±0.09ab	4.30±0.06ab
T_4	2.87±0.23b	3.07±0.24b	3.23±0.23b	3.37±0.24b	3.37±0.20b	3.47±0.20b
T_5	4.37±0.67a	4.53±0.68a	4.73±0.64a	4.87±0.62a	4.93±0.64a	5.00±0.61a
TREATMENTS	**10 WAS**	**11 WAS**	**12 WAS**	**13 WAS**	**14 WAS**	
T_1	3.57±0.61b	3.63±0.56b	3.73±0.56b	3.87±0.55b	3.87±0.56b	
T_2	4.07±0.35ab	4.13±0.38ab	4.27±0.39ab	4.30±0.40b	4.30±0.40b	
T_3	4.40±0.06ab	4.43±0.07ab	4.50±0.06ab	4.53±0.07ab	4.53±0.07ab	
T_4	3.50±0.23b	3.57±0.20b	3.67±0.20b	3.67±0.20b	3.67±0.20b	
T_5	5.13±0.60a	5.30±0.57a	5.47±0.50b	5.67±0.49a	5.67±0.49a	

T_1 = 100% w/v crude extract of *Tithonia diversifolia*; T_2 = 50% w/v extract of *Tithonia diversifolia*; T_3 = 25% w/v extract of *Tithonia diversifolia*; T_4 = 12.5% w/v extract of *Tithonia diversifolia*; T_5 = Control: equivalent amount of water; WAS = Weeks after sowing. Mean ± SEM (n=5). The columns with the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

Table 6 showed theeffects of treatment on leaf area of *G. max.* The effects of the treatment on areas of the leaf of *G.max* was significantly higher ($P \leq 0.05$)among the treatments from 4 to 14 WAS. This implies that the groups of soybean stands treated with water were observed to have larger area than the other treatments. At4WAS, the control (T_5) showed higher significant mean values in the area of their leaves than groups of the soybeans treated with treatment T_1(22.76±7.1cm^2), T_2(34.42±7.3cm^2), T_4(30.67±4.29cm^2), whose effects on leaf area of soybeans were more significantly lower than T_3 (37.79±1.76cm^2). This similar trend was observed among treatments from week 5 to 9 after sowing. At 10 and 11 WAS, the order of the significant effect of the treatment on the leaf area were T_5>T_3> T_2,> T_1, T_4. There was a significant reduction in the leaf area of the soybean. This observation was consistent with Patterson (1981) who detected that the application of some synthetic allelochemical reduced the leaf area of soybeans.

Table 6: Effect of the treatments on the leaf area (cm^2)

TREATMENTS	4WAS	5 WAS	6 WAS	7 WAS	8 WAS	9 WAS
T_1	22.76±7.14b	28.28±8.58b	36.33±11.43b	39.58±11.38b	42.14±11.78b	43.34±11.76b
T_2	34.42±7.32b	41.90±9.21ab	48.14±9.62ab	60.25±15.85ab	62.85±16.47ab	65.90±16.11ab
T_3	37.79±1.76ab	44.96±1.79ab	60.76±4.29ab	65.54±5.30ab	67.12±4.30ab	68.97±3.34ab
T_4	30.67±4.29b	37.18±5.49b	41.35±6.18b	44.87±6.81ab	46.07±6.28ab	48.64±6.70ab
T_5	61.51±14.74a	71.62±17.12a	78.32±18.28a	81.26±17.76a	84.25±17.87a	86.30±17.50a
TREATMENTS	**10 WAS**	**11 WAS**	**12 WAS**	**13 WAS**	**14 WAS**	
T_1	46.51±11.54bc	48.40±10.92bc	50.54±10.63bcd	53.92±10.23bc	53.92±10.25bc	
T_2	68.59±15.06abc	71.37±15.31abc	75.66±14.76abc	76.98±14.30b	76.98±14.30b	
T_3	72.02±2.64ab	74.63±2.50ab	76.95±2.42ab	79.02±2.05b	79.02±2.05b	
T_4	50.32±7.27bc	52.67±7.19bc	56.01±6.63bcd	57.67±6.18bc	57.67±6.18bc	
T_5	89.99±18.01a	96.79±16.93a	103.79±14.88a	110.26±13.32a	111.59±13.46a	

T_1 = 100% w/v crude extract of *Tithonia diversifolia*; T_2 = 50% w/v extract of *Tithonia diversifolia*; T_3 = 25% w/v extract of *Tithonia diversifolia*; T_4 = 12.5% w/v extract of *Tithonia diversifolia*;
T_5 = Control: equivalent amount of water; WAS = Weeks after sowing. Mean ± SEM (n=5). The columns with the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

Table 7 showed the effects of treatment on the production of soybean. The mean values of pods produced per stand among all the treatments were observed to be significantly the same.

Table 7: Effects of treatments on the mean numbers of pods per stand (yield)

TREATMENTS	NO OF POD
T_1	$12.33±0.33^a$
T_2	$17.33±10.35^a$
T_3	$16.67±6.67^a$
T_4	$12.33±2.85^a$
T_5	$14.00±1.53^a$

T_1 = 100% w/v crude extract of *Tithonia diversifolia*; T_2 = 50% w/v extract of *Tithonia diversifolia*;
T_3 = 25% w/v extract of *Tithonia diversifolia*; T_4 = 12.5% w/v extract of *Tithonia diversifolia*; T_5= Control: equivalent amount of water; WAS = Weeks after sowing. Mean ± SEM (n=5). The columns with the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

Table 8 showed that weight of the seed produced among the treatment were not significantly different ($P \leq 0.05$) from one another. The dry weights of the empty pods were measured to be the same in the mean values of their level of significance. There was no significant different ($P \leq 0.05$) in dry weight of the plant among the treatments. The effects of the treatments on the biomass which was considered as the weighed mass of the dried seed (g) plus dry mass of the empty pods plus the mass of the whole plant [shoot +leaves + root] (g) were significantly the same. Although, their mean value differs from one another among the treatments with T_3 (1220±4.02g) showing the highest value the means values were said to be statistically the same at $P \leq 0.05$ using Duncan's Multiple Range Test.

Table 8: The effects of treatment on dry weight of plant (biomass) (g)

TREATMENT	(A)	(B)	(C)	BIOMASS (A+ B +C)
T_1	3.32±0.43[a]	1.62±0.18[a]	2.71±0.39[a]	7.66±1.00[a]
T_2	4.49±2.09[a]	3.21±1.89[a]	4.21±1.72[a]	5.69±1.91[a]
T_3	5.02±1.52[a]	2.81±1.21[a]	4.37±1.30[a]	12.20±4.02[a]
T_4	3.01±0.24[a]	1.52±0.09[a]	2.91±0.31a	7.44±0.64[a]
T_5	5.01±0.37[a]	2.27±0.19[a]	1.77±0.59[a]	9.06±1.10[a]

T_1 = 100% w/v crude extract of *Tithonia diversifolia*; T_2 = 50% w/v extract of *Tithonia diversifolia*; T_3 = 25% w/v extract of *Tithonia diversifolia*; T_4 = 12.5% w/v extract of *Tithonia diversifolia*; T_5 = Control: equivalent amount of water; WAS = Weeks after sowing. Mean $\pm$ SEM (n=5). The columns with the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test. A = Dry weight of seed, B = Dry weight of empty seed pod, C = Dry weight of whole plant (shoot + leaves + root).

Conclusion

Glycine max was susceptible to the phytotoxic activity of leaf water extract from *Tithonia diversifolia*. *Tithonia diversifolia* showed high significant phytotoxicity effect on the crop. This study showed that *Tithonia diversifolia* was an allelopathic weed with water-soluble allelochemicals in its plant parts and had such phytotoxic potency that could suppress the growth of associated crop plants. The use of extract from the leaves of *Tithonia diversifolia*as alternative organic booster for cultivars of *G. max* may not be a wise decision.

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