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Physiological Response of Hibiscus Sabdariffa to Multi-Year Rainfall-Leached Allelochemicals of Tithonia Diversifolia and Chromolaena Odorata

A. A. Ogunwole
Lecturer, Department of Biological Sciences, Wesley University of Science and Technology, Ondo, Nigeria
O. O. Otusanya
Plant Physiologist, Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria
F. A. Oloyede
Senior Lecturer, Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria
M. O. Tijani
Ph.D. Student, Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract:
The awareness of conditions under which fallowed land stimulate the general growth and yield of subsequent crops would enable us to effectively manage the land and take advantage of the growth-enhancing potential of the leachates of fallowed weeds. A four-year experiment was conducted under natural condition to explore and compare the effects of one, two and three year's accumulated leachates plus exudates of Chromolaena odorata or Tithonia diversifolia on the growth, calyx yield, accumulation of chlorophyll, ascorbic acid and protein in Hibiscus sabdariffa crop. Seedlings of H. sabdariffa were grown on the soil which had received one, two and three year's aqueous-leachate plus exudates of C. odorata or T. diversifolia. Results show that all the studied parameters were enhanced by the leachates plus exudates of both weeds in individual year. However, optimum enhancement of these parameters was induced by two years accumulated leachates plus exudates while the three years accumulated leachates plus exudates induced least enhancement. Generally, the study presents the leachates plus root exudates of these weeds as a good source of cheap and highly effective crop-growth enhancer. It also recommends the accumulated two years leachates plus exudates or a piece of land dominated with these weeds and fallowed for two years for enhancing and optimizing the production of H. sabdariffa. Accumulation of ascorbic acid could be the adaptive mechanisms evolved by H. sabdariffa to allelopathic tolerance. Screening for the active ingredients in these weeds leachates and/or exudates is therefore suggested so as to produce crop-growth enhancer or regulators.

Keywords: *Allelochemicals, Calyx Yield, Chromolaena odorata, Exudates/Leachates, Tithonia diversifoli Multi-year*

1. Introduction

The ever-increasing human population has resulted in rapid urbanization of vast expanse of agricultural lands; consequently, the food security across the world especially in the developing countries is under threat. There is a need to explore several strategies for combating the menace of hunger in these parts of the world. One common practice by peasant farmers in the developing countries is bush fallowing. Fallowing a piece of land for 2 or 3 years replenishes the soil with its deprived nutrients. Studies show that this practice is one of the environment-friendly ways of maintaining soil fertility, increasing crop yield and controlling weed in the humid and sub humid tropics (Kang *et al.,* 1990; Akobundu *et al.,* 1992). Bush fallow practice helps in the regeneration of soil fertility, reduction of soil erosion and leaching, maintenance of soil physical and biological condition, accumulation of biomass, green manures and other properties which determine partially the growth and yields of crops. However, factors such as vegetation composition, fallow duration/age, presence of indicator plants and earthworm casts have been implicated as the indicators of the fertility of a particular fallowed land. Studies indicated fallow duration/age as the most important indicator of fertility in fallow practice (Norgrove and Hauser, 2015). According to Guillemin (1956) and Mertz (2002), there is an optimum fallow period at which agricultural production is enhanced since in every agrosystem, shorter fallow periods lead to a decline in productivity while longer fallow period is quite unnecessary.

Tithonia diversifolia (Hemsl) A. Grayand *Chromolaena odorata* L. King and Robinson are the prevalent and predominant allelopathic weeds in Southwest Nigeria. They belong to the family Asteraceae and are ranked excellent fallow species. The soil on which these weeds grow have higher pH, porosity, moisture content, N, P, K, Na, Ca, mycorrhizal fungi spores, earthworm cast density and lower bulk density (Atayese and Liasu, 2001). Also, they are able to extract relatively high amount of nutrients from the soil and contain relatively high nutrient concentrations in their biomass, thus, have the potential of being used as nutrient source [Akanbi and Ojeniyi, 2007; Agbede *et al.,* 2014]. These potentials have led to attribution of the stimulatory effects of these species to high inorganic nutrient concentrations in their biomass and rapid decomposition of their litter fall by the previous studies. However, Ries *et al.,* (1977) argued that the highly significant increase in the growth and total N content of tomato, cucumber and lettuce was not a product of high mineral contents only but a stimulating factor (allelochemicals) present in chopped Alfalfa must have contributed. Similarly, Rice, (1987) stated that the applied 2g dry residue of ground-ivy (Glechomahederacea) per Kg of soil couldn't have remarkably promoted the radish growth without external aids. The author suggested the aid to be an allelochemical(s) capable of increasing the uptake of minerals by test plant in the ground-ivy extract. Neil and Ries (1971) quoted by Rice (1986) found that rhizosphere soil from western ragweed (*Ambrosia psilostachya*) markedly stimulated the growth of ten (10) plant species, 2 species, 4 species and another 4 species of these ten species were later reportedly enhanced respectively by the root exudates, leaf leachates and dried leaves of this weed. In fact, De-Albuquerque *et al.,* (2011) also argued that plant-crop allelopathic interaction plays essential role in the replenishment of soil fertility especially when method such as crop rotation, bush fallow, use of green manure, minimal tillage, use of cover crops etc. is practiced. Also, Jabran *et al.* (2012) opined that allelochemicals play key role in crop nutrient management through biological nitrification inhibition (BNI) and nutrient acquisition through solublization, nutrient uptake and nutrient retention.

It is an established fact that under natural conditions, substantial amount of allelochemicals are leached out of the aerial parts (stem, leaves, flowers and seed) of environmental weeds through rain-wash and exudation into the soil (Tongma *et al.,* 1998; Taiwo and Makinde, 2005; Otusanya *et al.,* 2008). These hydrophilic compounds are potential enhancer of germination, growth and physiological parameters of the neighbouring or successional crops (Ambika and Poornima, 2004; Aladejimokun *et al.,* 2014).Also, they function as promoting agent for tissue formation, cell division and enlargement processes etc., thereby regulating both the production and activities of plant hormones (Farooq *et al.,* 2013). However, the composition, efficacy, quantity and activities of allelochemicals in the soil depend on the prevailing environmental conditions, soil physical and chemical composition, diversity and activities of soil biota and the donor/recipient status (Dalton, 1999; Okumura *et al.,* 1999; Otusanya *et al.,* 2015). Literature review showed that there is dearth of scientific data on the net effects of the fallowed weeds leachates plus root exudates on the growth and yield of subsequent crop(s). Also, little attention has been given to the time frame required for the accumulation or degradation of these leachates/exudates in the soil and the possible effect of this duration on subsequent crops. The latter is essential because the concentration range between the enhancement and inhibition induced by allelochemicals is narrower for some crop species and broader for others. This study therefore, under natural condition, explores and compares the effects of one, two and three years' leachates of *T. diversifolia* and *C. odorata* on the growth, calyx yield, chlorophyll, ascorbic acid and crude protein contents of *H. sabdariffa.*

2. Materials and Methods

2.1. Experimental Site

The experiment was carried out at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria between 2009 and 2012. Ile-Ife is situated between $7^{0}33^{I}$N and $4^{0}32^{I}$E. The area is characterized with bimodal rainfall with a record of 1445 mm, 1510 mm, 1312 mm and 1320 mm mean annual rainfall in the year 2010, 2011 and 2012 respectively. The temperature in Ile-Ife during these seasons varied from 27±4°C during the day to 22±3°C at night with 10-12 hours' photoperiod. Relative humidity, wind speed and evaporation rate were also remained more or less similar during these years (ARCSSTE-E), Ile-Ife).

2.2. Materials Sources

The seeds of *H. sabdariffa* and *T. diversifolia* were collected from National Horticultural Research Institute (NIHORT) Ibadan and along Ede road, near the O.A.U. main campus gate respectively. Young seedlings of *C. odorata* were collected from the site of the Botany Department Afforestation Scheme, O.A.U. Ile-Ife.

2.3. Seeds and Seedlings Treatments

H. sabdariffa and *T. diversifolia* seeds were randomly selected and soaked for five minutes in 5% sodium hypochlorite. Thereafter, the seeds were rinsed in running tap water for 5 minutes and then thoroughly washed in double distilled water. About 200 seedlings of *C. odorata* were randomly sorted out on the basis of vigour and height.

2.4. Soil Culture Experiment

In June 2009, 450 experimental pots (55 cm diameter × 75 cm depth) were filled with homogenous top humus soil and divided equally into Plots A, B and C. Five surface sterilized seeds of *T. diversifolia* were sown in each of the pots in plot A while three seedlings of *C. odorata* were planted in each of the pots in plot B. No weed was allowed to grow in the pots in plot C and these were allocated as the control (CTR). Each experimental pot had six holes perforated at the bottom for good drainage and were arranged in a complete randomized block design. At two weeks, the plants in each pot (plot A and B alone) were thinned down to two uniform plants. Weeding and removal of pests were carried out on a daily basis. Decomposition of senescent leaves of the weedsin the potted soil was completely prevented to ensure that only the rain-leached products plus root exudates of the particular weed in a plot account for the difference observed.

In June 2010, the potted weeds in plot A and B were randomly selected, divided equally into three and allocated as follows; One-year leachate (2010 season), two years' leachate (2011 season) and three years' leachate (2012 season). In other words, the soil in plot A had received one year, two years and three years leachates plus exudates of *T. diversifolia* by June 2010, June 2011 and June 2012 respectively whereas aqueous soluble products in aerial parts plus root exudates of *C. odorata* had thoroughly mixed with the soil in plot B for one, two and three years by June 2010, 2011 and 2012.Thereafter, all the ascribed2010 season experimental weeds in plot A and B were carefully uprooted from the pots and the soil in each pot was thoroughly mixed for maximum aeration. Pots removed from plot A were given the subscripts; *T. diversifolia* Water Leachates plus exudates (2010TWL) while pots removed from plot B were ascribed *C. odorata* Water Leachates plus exudates (2010CWL). Fifty pots were also removed from plot C and were given the subscript 2010 control (2010CTR).

2.5. Sowing and Harvesting of H. sabdariffa

Ten surface sterilized seeds of *H. sabdariffa* were sown in each of the 2010 experimental pots. The pots arranged in a complete randomized design were then placed in an open space in front of Botany Department, Obafemi Awolowo University, Ile-Ife, Nigeria. At two weeks, the seedlings in each pot were thinned down to six uniform plants per pot and harvesting of the plants commenced immediately. Thereafter, harvesting was carried out on a weekly basis.

2.6. Recording of Data

Measurements of the following growth parameters (shoot height, stem girth, number of leaves) were carried out according to the standard methods and the data recorded. The leaf area was determined using the method of Pearcy *et al.,* (1989) and the leaf area ratio calculated. Five shoots in each regime were weighed on Mettler Toledo balance (PB203) to obtain the fresh weight. The shoots were then packaged separately in envelopes and dried to constant weight at 80^0C in a Gallenkhamp oven (Model IH-150) to obtain the dry weight. Chlorophyll contents of the fresh shoot were extracted with 80% acetone and quantified following the procedure of Comb *et al.,* (1985). Ascorbic acid and protein contents were determined according to the titrimetric method and micro-Kjeldahl nitrogen method respectively as described by AOAC (2000). Ascorbic acid in the sample was estimated using the formula below:

$$\text{Ascorbic acid in the Sample} = \frac{\text{X} \times \text{Y} \times \text{Titre of Sample}}{gramofSample}$$

X = the ascorbic acid quantity equivalence of 1ml dichloro-indolephenol

Y = the ratio of the quantity (mL) of the extraction solution used for extracting the sample to the quantity (mL) taken for titration.

The percentage crude protein accumulation in the shoot of *H. sabdariffa* was estimated using the formulae below.

$$\%\ \text{Total Nitrogen} = \frac{(A - B) \times N \times 14.01 \times 100}{\text{gram of Sample} \times 10}$$

% Crude Protein = % Total Nitrogen × 6.25

A = sample reading, B = blank reading; N = Normality of acid used for titration, 100 = conversion to % and 6.25 is the correction factor (F).

Eighteen weeks after planting (18WAP), the calyces on twenty randomly selected plants per regime were counted and the mean recorded. Each calyx was weighed immediately on Mettler Toledo (PB153) balance to obtain the fresh weight. Thereafter, the calyces were then packaged separately in envelopes and dried to constant weight at 80^0C in a Gallenkhamp oven (Model IH-150) to obtain the dry weight.

2.7. 2011 and 2012 Experiments

Adequate care was given to the remaining experimental pots in each plot until they were utilized in 2011 and 2012 season ascribed to them. By June 2011, the experimental pots in plot A and B had received two consecutive years' leachates of *T. diversifolia* and *C. odorata* respectively. Similarly, by June 2012, the pots in each plot had received three years' consecutive leachates of the respective weed in the plot. All the experiments carried out in 2010 season were therefore reproduced in 2011 and 2012 seasons except that the pots removed from plot A and B these seasons were given the subscripts 2011TWL, 2012TWL and 2011CWL, 2012CWL respectively.

2.8. Statistical Analysis

All experiments were conducted in five replicates and the data obtained were subjected to analysis of variance (ANOVA). Differences between individual means were determined by least significant difference (LSD) test at 0.05 level of probability. Data were analyzed using SPSS.

3. Results

3.1. Growth Characteristics

Figures 1 to 10 below show the effects of *T. diversifolia* (TWL) and *C. odorata* (CWL) leachates on the shoot height, root length, number of leaves, stem girth, leaf area, shoot fresh and dry weights, root fresh weights and biomass of *H. sabdariffa* plants in 2010, 2011 and 2012 seasons. Generally, one (2010), two (2011) and three (2012) year's accumulated TWL and CWL enhanced all the above growth parameters with the peak increase recorded two years accumulated leachates. The enhancement of these growth parameters followed the trend: 2011CWL > 2011TWL > 2010CWL > 2012CWL > 2010TWL > 2012TWL. This indicates that under the same environmental conditions, the CWL could enhance the vegetative growth of *H. sabdariffa* than TWL. Multi-year statistical comparison show that accumulated two years' leachates of TWL and CWL significantly enhanced all the growth parameters studied compare with one and three years' leachates at $p<.05$. In other words, all the growth parameters studied were remarkably enhanced in 2011 season than 2010 and 2012 season at $p<.05$.

3.2. Chlorophyll Pigments

The levels of chlorophyll pigments in the treated *H. sabdariffa* were greater than that of the control in the three seasons (Figures 11-13). Like the growth parameters, the stimulation of chlorophyll pigments by CWL and TWL during the three seasons followed the order: 2011CWL > 2011TWL > 2010CWL > 2012CWL > 2010TWL > 2012TWL. Also, compared with the CTR, chlorophyll a and total chlorophyll accumulation in *H. sabdariffa* were significantly enhanced by two years (2011) accumulated leachates of both weeds at $p<.05$. However, increase induced by one and three years' leachates of these weeds were insignificant at $p<.05$. Multi-year statistical analysis showed that accumulated chlorophyll a and total chlorophyll (in treated *H. sabdariffa*) in 2011 season were significantly greater than those recorded in 2010 and 2012 seasons at $p<.05$. The indication is that the two year accumulated leachates of both weeds were more effective in enhancing the chlorophyll pigments in this crop.

3.3. Bio-molecules Accumulation

The effects of accumulated one, two and three years TWL and CWL on the contents of ascorbic acid and protein in *H. sabdariffa* are as shown in Figures 14 and 15. Just like the growth parameters, the CTR and CWL plants recorded respectively the least and highest values of both biomolecules. Also, multi-seasons comparison showed that the least and optimum enhancement were induced by three (2012TWL) and two (2011CWL) years leachates respectively. The levels of these bio-molecules in two and three-years-CWL-treated plants were significantly greater than the CTR at $p<.05$ whereas significant increase was recorded for only two-years-TWL-treated plants at $p<.05$. Statistically, the ascorbic acid and protein levels (in treated *H. sabdariffa*) in 2011 season were significantly higher than those of 2010 and 2012 seasons at $p<.05$.

3.4. Calyx Yield

The effects of CWL and TWL on the number of calyces, fresh and dry weights of *H. sabdariffa* plants are as shown in Figures 16, 17 and 18 respectively. Eighteen weeks after planting, the plants grown on 2011CWL-treated soil produced the highest number as well as the greatest fresh and dry weights of calyx. However, the 2012CTR-grown plants had the lowest values. These parameters in the treated plants were significantly greater than those of the control in each season. Furthermore, the plants grown on two years accumulated leachates recorded significantly higher values for these parameters compare with one and three years accumulated leachates-treated plants at $p<0.05$. The results indicated that leachates of these weeds can enhance the growth of *H. sabdariffa* plant up to the reproductive stage. It also showed that two years accumulated leachates are better enhancer of the yield of calyces than one or three year's accumulation.

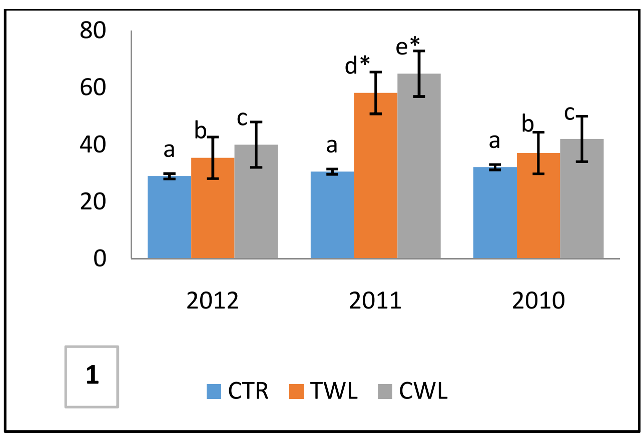

Figure 1

Effects of accumulated one, two and three year's Leachates plus exudates of *T. diversifolia* (TWL) and *C. odorata* (CWL) on Shoot Height of *H. sabdariffa*[1]

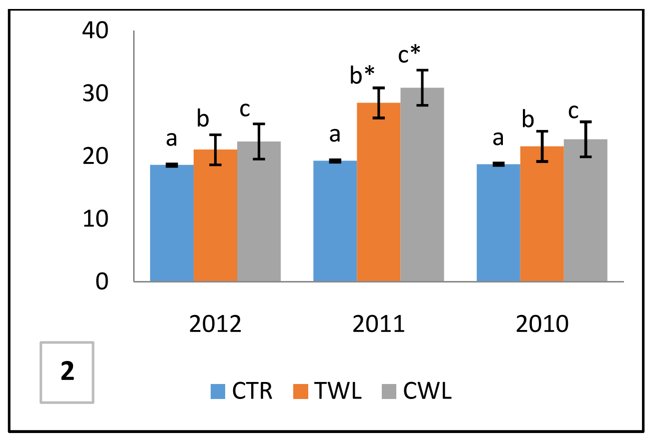

Figure 2

Effects of accumulated one, two and three year's Leachates plus exudates of *T. diversifolia* (TWL) and *C. odorata* (CWL) on Root Length of *H. sabdariffa*

[1] In these chats and the chats to follow, *represents mean significantly different from CTR at $p<0.05$; Mean of different years/seasons followed by the same letter are not significant at $p<0.05$; Vertical Bars represent the error bars with standard error

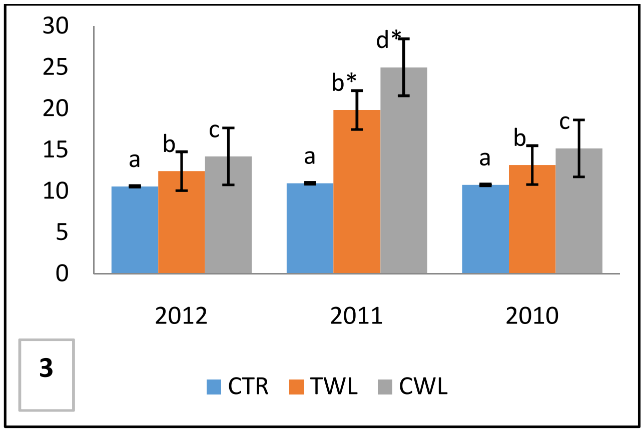

Figure 3

Effects of *T. diversifolia* (TWL) and *C. odorata* (CWL) leachates plus exudates on number of leaves on *H. sabdariffa* in 2010, 2011 and 2012 Seasons

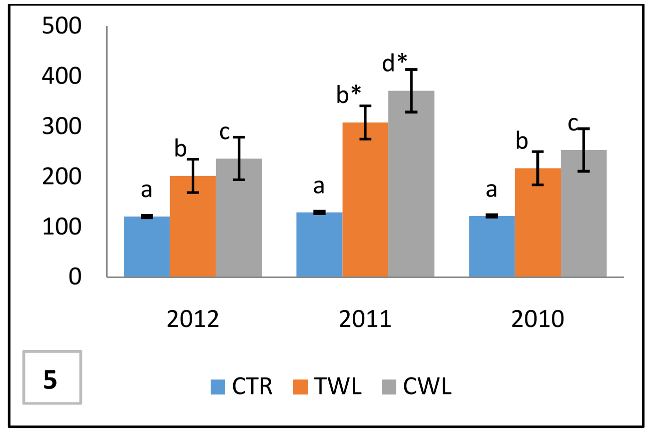

Figure 5

Effects of *T. diversifolia* (TWL) and *C. odorata* (CWL) leachates plus exudates on the Leaf Area of *H. sabdariffa* in 2010, 2011 and 2012 Seasons

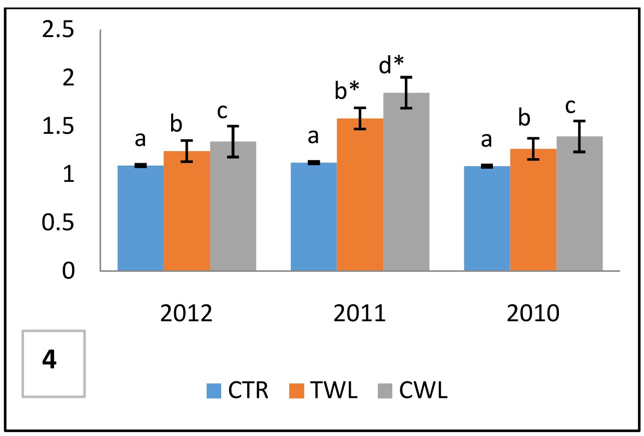

Figure 4

Effects of *T. diversifolia* (TWL) and *C. odorata* (CWL) leachates plus exudates on Stem Girth (5) of *H. sabdariffa* in 2010, 2011 and 2012 Seasons

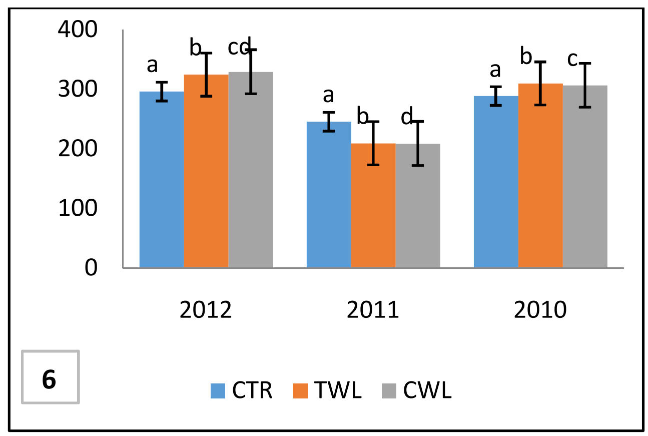

Figure 6

Effects of *T. diversifolia* (TWL) and *C. odorata* (CWL) leachates plus exudates on Leaf area Ratio of *H. sabdariffa* in 2010, 2011 and 2012 Seasons

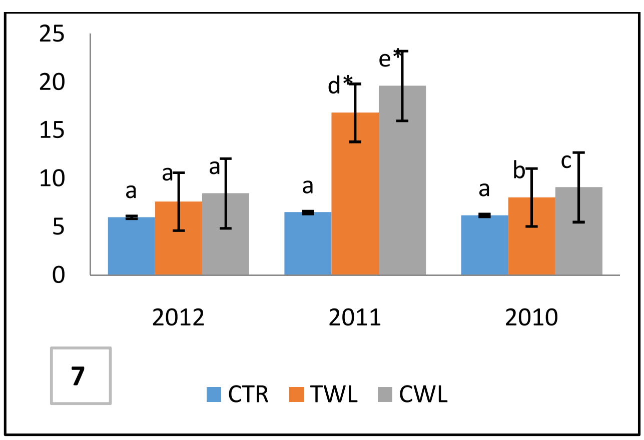

Figure 7

Variations in the shoot fresh weight as effected by one, two and three year's accumulated leachates plus exudates of *T. diversifolia* and *C. odorata*

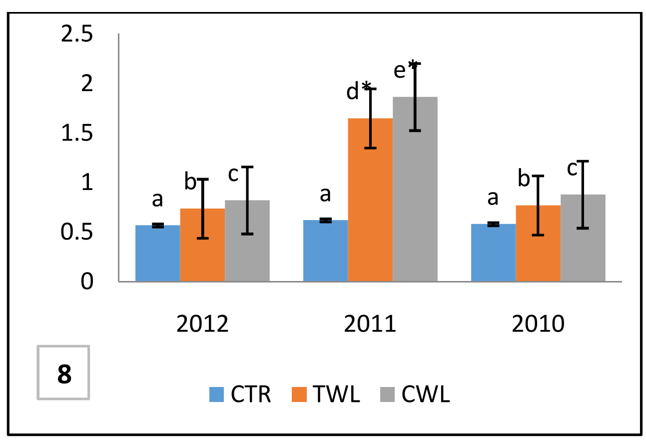

Figure 8

Variations in Shoot Dry Weight as effected by one, two and three year's accumulated leachates plus exudates of *T. diversifolia* and *C. odorata*

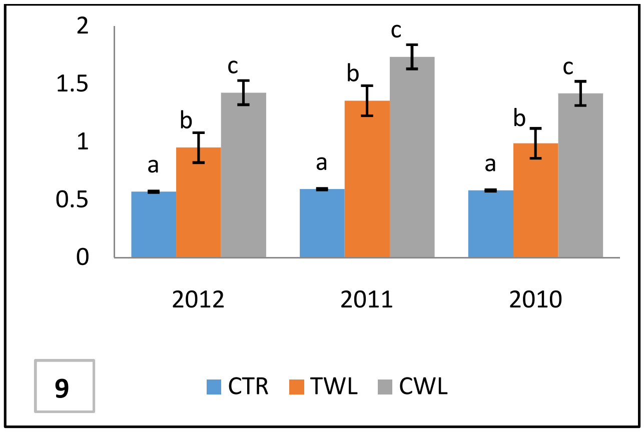

Figure 9

Variations in the Root fresh weight and as effected by one, two and three year's accumulated leachates plus exudates of *T. diversifolia* and *C. odorata*

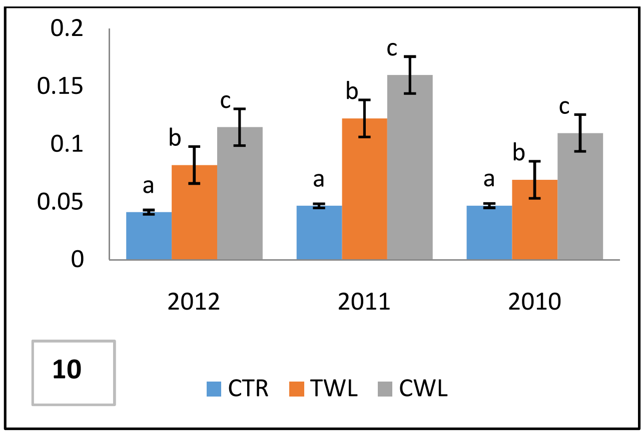

Figure 10

Variations in the Root biomass as effected by one, two and three year's accumulated leachates plus exudates of *T. diversifolia* and *C. odorata*

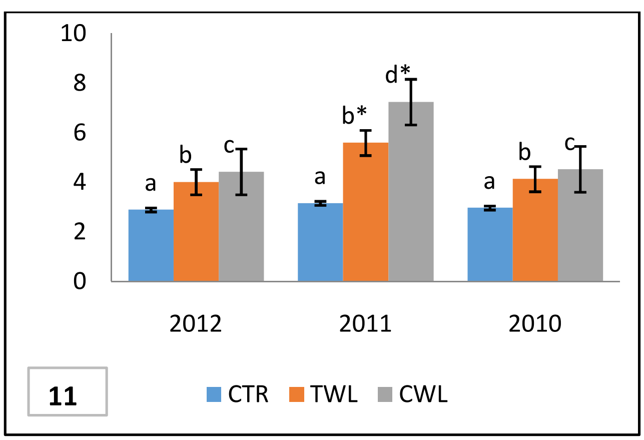

Figure 11

Changes in the levels of Chlorophyll a in *H. sabdariffa* plant as effected by one, two and three years accumulated leachates of *T. diversifolia* and *C. odorata*

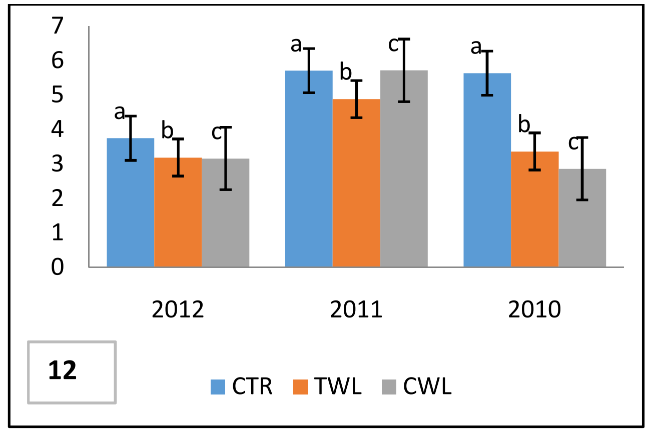

Figure 12

Changes in the levels of Chlorophyll b in *H. sabdariffa* plant as effected by one, two and three years accumulated leachates of *T. diversifolia* and *C. odorata*

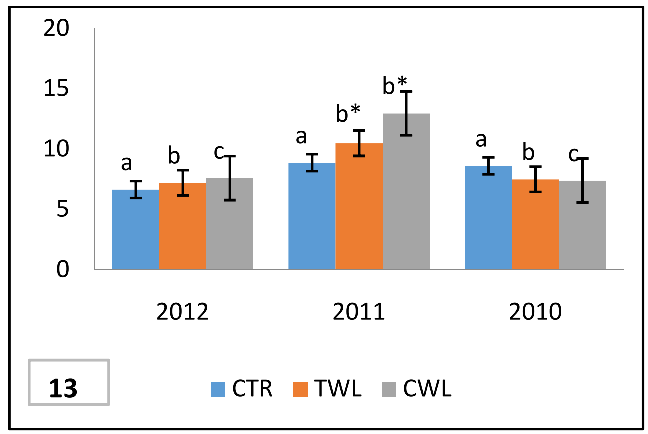

Figure 13

Changes in the levels of Total Chlorophyll in *H. sabdariffa* plant as effected by one, two and three years accumulated leachates of *T. diversifolia* and *C. odorata*

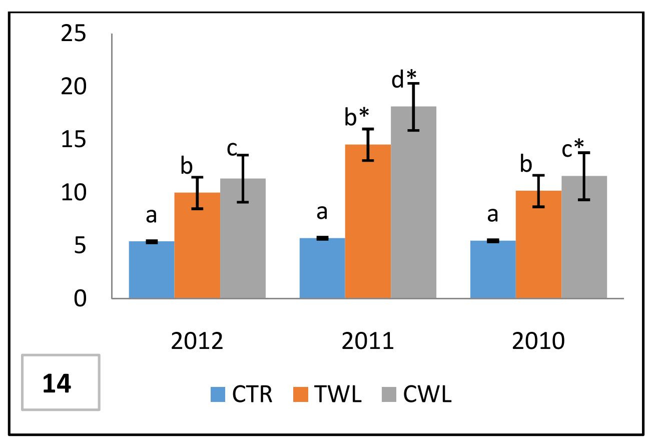

Figure 14

Changes in the levels of Ascorbic acid in *H. sabdariffa* plant as effected by one, two and three years accumulated leachates of *T. diversifolia* and *C. odorata*

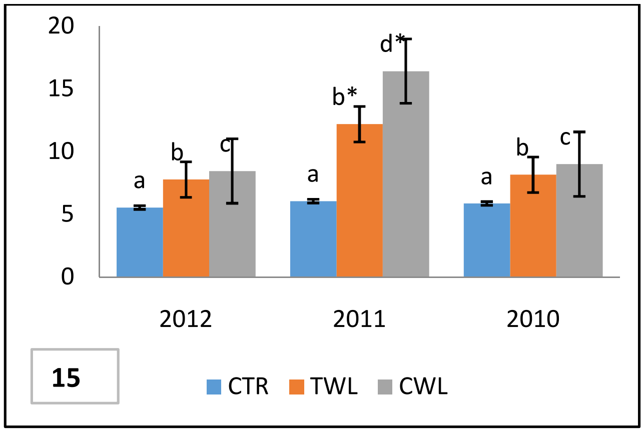

Figure 15

Seasonal Changes in the Protein percentage of *H. sabdariffa* plant as effected by one, two and three years accumulated leachates plus exudates of *T. diversifolia* and *C. odorata*

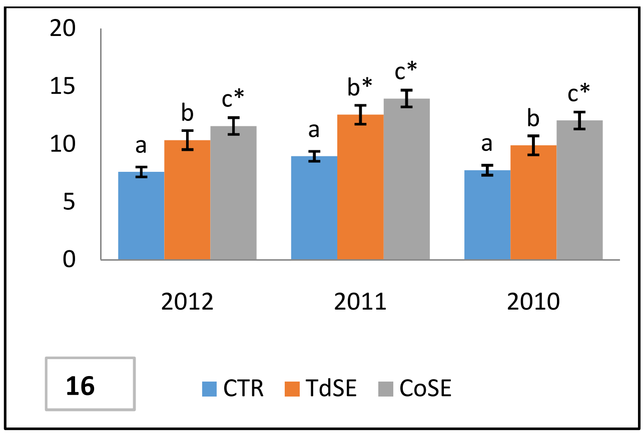

Figure 16

Seasonal Changes in the Number of calyces of *H. sabdariffa* plant as effected by one, two and three years accumulated leachates plus exudates of *T. diversifolia* and *C. odorata*

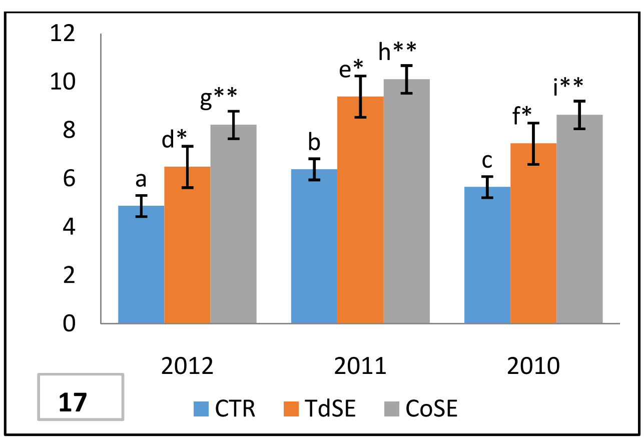

Figure 17

Seasonal Changes in the Calyx fresh weight of *H. sabdariffa* plant as effected by one, two and three years accumulated leachates plus exudates of *T. diversifolia* and *C. odorata*

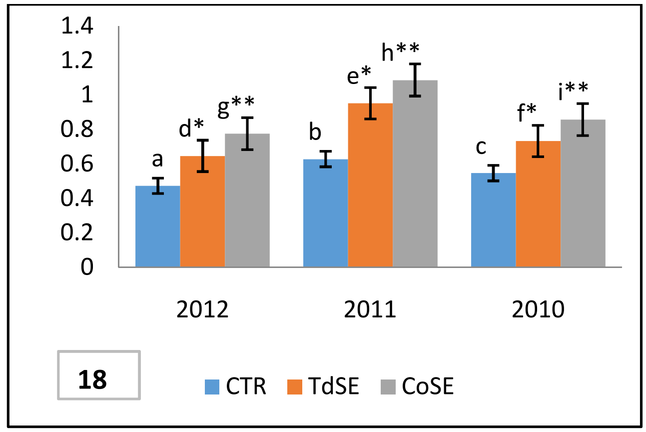

Figure 18

Seasonal Changes in the Calyx Dry Weight of *H. sabdariffa* plant as effected by one, two and three years accumulated leachates plus exudates of *T. diversifolia* and *C. odorata*

4. Discussion

Allelochemicals in the weeds leachates have direct as well as indirect effects on plants. The different physiological and biochemical changes effected in growth metabolism of plants constitutes the direct effects while the indirect effects include alteration in soil physiochemical properties, change in microbial populations and differential nutrient availability to plants (Rizvi and Rizvi,1987). Inderjit and Duke, (2003) stated that allelochemicals in the weeds-leachates stimulate the growth of crops by either altering the soil chemistry or the soil biota population and activities. In this study, the leachates of *T. diversifolia* (TWL) as well as *C. odorata* (CWL) significantly enhanced the vegetative growth of *H. sabdariffa* probably by improving and maintaining the crop-growth-promoting qualities of the soil such as the soil pH, porosity, moisture content and increased the mycorrhizal fungi spores, earthworm cast density and bulk density of the soil. Moreover, virtually all allelochemical interactions involve the sum total of the leachates and exudates of higher plants and those of micro-organisms (Putnam 1985). This author argued that the aggregate of the leachates and exudates of plants are usually agents of crop-growth enhancer or detoxifiers of phytotoxicchemicals or activity of allelopathy. It could therefore be said that the enhancement of *H. sabdariffa* growth in this study is a product of both the leachates of the aerial parts and the root exudates. In fact, the composition of soluble plant root exudates (mixtures of sugars, amino acids, sugar alcohols, organic acids, phenolics, vitamins, proteins, mucilage carbon IV oxide, aldehydes and various secondary metabolites) reportedly count for 10-40% of the total carbon fixed during photosynthesis (Bais *et al.,* 2006). In addition, Singh and Mukerji (2006) and Neumann (2007) categorically described these organic substances (root exudates) as important drivers of microbial and fauna activity in soil due to their relatively high bioavailability, their role in controlling the bioavailability of nutrients (e.g., phosphorus) and phytotoxic elements (e.g., aluminium), together with the fact that they are added to soil on a regular/semi-continuous basis. One can therefore assume that the root exudates of *T. diversifolia* (TWL) as well as *C. odorata* (CWL) served as the carbon source or the precursors for the synthesis of soil humus, thereby enhancedthe availability and acquisition (uptake and transport) of nutrients by *H. sabdariffa.* Perhaps, the soluble organic acids such as malic, oxalic and citric acid contained in the root exudates of these weeds facilitated the *H. sabdariffa* plant root-rhizobium infestation or functioned as carbon sources for microbial nutrition. In general, this study presents the leachates of the aerial parts plus the root exudates of these weeds as a good source of cheap and highly effective crops-growth enhancer. The results corroborate the findings of Ambika and Poornima, (2004), Oyerinde *et al.,* (2009), Ilori *et al.,* (2011), Aladejimokun *et al.* (2014) and Otusanya *et al.,* (2015) who observed significant increase in the vegetative growth of cluster bean, soybean, radish, ragi plants, *Zea mays*, *Celosia argentea* and *H. sabdariffa* plant treated with the extracts of either *T. diversifolia* or *C. odorata*.

The effects of allelochemicals on crops can be stimulatory or inhibitory depending on the identity of the active compound as well as the persistence and fate of organics in the environment and on the particular target species (Inderjit and Keatin, 1999). In this study, the establishment of the seedlings as well as optimum vegetative growth of *H. sabdariffa* was rapid and effective in TWL and CWLexperimental pots, however, the persistence in the soil, of some phytotoxic compounds in them reduced the growth and final yield of *H. sabdariffa*. The two years (2011) accumulated leachates of *T. diversifolia* (TWL) and *C. odorata* (CWL) significantly promoted the studied growth characteristics than one (2010) and three (2012) years' leachates with the three years' accumulated leachate recorded the least enhancing effect. This indicated that the concentration of the TWL or CWL accumulated in the soil for two years was not enough to cause significant inhibition of the growth of the test crop; rather, it was enhanced. However, through accumulation, the concentration of the phytotoxic allelochemicals in CWL and TWL peaked after two years, and eventually became toxic to *H. sabdariffa* plant. These results suggested the following; first, the degradation of some active allelochemicals to the reduced/ detoxified and absorbable forms by microbial activities during the first two seasons. Second, the adsorption in the soil of some phytotoxic allelochemicals in the leachates for more than two years which were desorbed and elicited their negative effect on the test crop in the third year. According to Kobayashi (2004), the concentration of allelochemicals effective to elicit negative impacts on agricultural crops is high and may not occur in fields due to soil factors such as adsorption, desorption and degradation by microbial activity. The persistence of plants leachates plus exudates for long period in the soil can lead to hyper-accumulation of these compounds in the environment and influence negatively the growth and development of the neighbouring and/or successional plants (Inderjit and Keating, 1999; Inderjit and Duke, 2003). In fact, Einhellig *et al.,* (1982) opined that certain concentration threshold exists for negative impact of leachates on crops below which might have stimulatory effect. Oudhia *et al.* (1999) also emphasized the fact that only the supra-optimal concentration of the leachates caused inhibition of crops growth on the field. These probably explain the remarkable diminution in the growth of *H. sabdariffa* induced by three years accumulated leachates plus exudates. The implication here is that the normal growth of *H. sabdariffa* does not only depend on the fallow weed species and target plants but also on the fallow periods/age.

The enhancement of the chlorophyll a and total chlorophyll in the shoot of treated-seedlings show that leachates of weeds (CWL and TWL in this case) can significantly alter the formation and accumulation of photosynthetic pigments in crops. Perhaps, CWL and TWL decreased the conversion of chlorophyll a to other photosynthetic pigments (carotenoids) or stimulate the formation of chlorophyll pigment precursors (porphyrin), enzymes (Mg-chelatase) and co factors (Cobalamin, Mg^{2+}, Fe^{2+}), thereby increased the accumulation of chlorophyll. This result corroborates the recent finding of Ogunwole and Otusanya (2015). The authors suggested the enhancement of the chlorophyll synthesizing system and/or the inhibition of chlorophyllase activity by the aqueous extracts of *T. diversifolia* and *C. odorata*. The significant increase in chlorophyll a and total chlorophyll recorded in 2011 season over the previous and subsequent seasons signified the greatest potency and activity of the two years' accumulated leachates of *T. diversifolia* or *C. odorata* on chlorophyll pigment formation over the one and three years' accumulated leachates. The implication of this result is that fallow age/duration does not only indicate soil fertility but could also determine the degree or levels of chlorophyll in the subsequent crops. This is consistent with the observed remarkable increase in the growth characteristics recorded in 2011 season. However, the significant reduction in growth and chlorophyll pigment recorded in 2012 seasons suggested the microbial transformation of some inactive phytotoxic allelochemicals to active ones or desorption of the accumulated phytotoxic allelochemicals in that season.

The results also show significant increase in the ascorbic acid levels of *H. sabdariffa* grown on CWL and TWL-treated soil. These increase may be the effect of flavonoids and phenolic compounds in the leachates which are precursors for the synthesis of anthocyanidin (flavonoids structure) and can potentially increase the anthocyanin contents of *H. sabdariffa.* Ziadi *et al.,* (2001) and Achuo *et al.,* (2004) reported that the foliar application of phenolic compounds derivatives (humic acid) consistently enhanced antioxidants such as α-tocopherol, α-carotene, superoxide dismutases and ascorbic acid concentrations in turf grass species. Enhanced levels of endogenous ascorbic acid in CWL and TWL-treated seedlings perhaps play several growth-promoting roles in *H. sabdariffa*. In other words, higher levels of endogenous ascorbic acid in these seedlings probablyconferred stress tolerance functions such as regulation of cell elongation and progression through the cell cycle (Smirnoff 1996; Horemans *et al.,* 2000), shielding of photosynthetic functions and other cellular components from oxidative damage, scavenging of other Reactive Oxygen Species (ROS) produced during photosynthesis and respiration (Asada and Takahashi 1987) etc. on the test crop. However, since ascorbic acid is an important co-factor in the biosynthesis of Salicylic acid, Ethylene, Jasmonic acid, Gibberellic acid and Abscisic acid and might even affect the levels and signaling of these hormones, the overall enhancement observed for this crop could have been through the alteration of the biosynthetic pathway of some of these hormones in the crop. It may therefore seem logical to trace or adduce the mechanism of action of CWL and TWL on *H. sabdariffa* to their remarkable influence on ascorbic acid accumulation in the crop.

In the case of protein, the increase in the *H. sabdariffa* shoot percentage protein could be the effect of CWL and TWL on increasing the nutrients availability and acquisition as well as alteration of the enzymatic and hormonal activities through production and degradation of various compounds in the test plants as suggested by Inderjit and Duke (2003). However, it could be due to interference of allelochemicals with the cytoplasmic ribosomes and production of RNA, which in turn stimulates protein synthesis. In fact, Inderjit and Nayyar (2002) noted that some allelochemicals are capable of stimulating protein synthesis via; increasing the incorporation of amino acid into protein, inducing the Abscisic acid or interfering with the cytoplasmic ribosomes and production of RNA. The significant increase recorded for protein in 2011 season could be the net effect of the enhanced growth and chlorophyll accumulation recorded in this year compare with previous and subsequent years. Bearing in mind the other factors, the result points to optimum fallow duration of two years or less (≤ 2) for enhancing the growth and nutritional quantities of *H. sabdariffa* especially when the land dominated with *T. diversifolia* or *C. odorata* is to be cultivated.

The aqueous leachates/ extract of both *T. diversifolia* (TWL) and *C. odorata* (CWL) are very rich in flavonoids (Otusanya and Ilori, 2012; Harini *et al.,* 2014). Therefore, the increase in the number, fresh and dry weights of calyces of *H. sabdariffa* grown in CWL and TWL experimental pots could be due to increase in the soil flavonoids which in turn stimulate the endogenous (*H.* sabdariffa) flavonoid contents. Ylstra *et al.,* (1992) and Schijlen *et al.,* (2007) found that flavonoids are essential for pollen development and pollen tube growth. In fact, the persistence for a long time of flavonoids in the soil perhaps enhanced the Arbuscular Mycorrhizal fungi in the soil, thus alters the soil physical, biological and chemical properties. Silva-Junior and Siqueira(1998) opined that flavonoids contained in the leachates could enhance the Arbuscular Mycorrhizal fungal growth via signaling and facilitating the encounters of host roots by the fungus. This result can be likened to the findings of Aulia *et al.,* (2009) who reported a remarkable increase in the number of fruit, fruit weight and calyx weight of *H. sabdariffa* treated with commercial product of mycorrhizal. However, the significant increase recorded in 2011 season for these parameters further emphasized the transient and ephemeral nature as well as the adsorption of virtually all the phytotoxic components of TWL and CWL in the soil. In other words, eighteen weeks after the weeds had been cleared off, the reported phytotoxic chemicals in the leachates of *T. diversifolia* and *C. odorata* either by accumulation or synergy formation had no significant effect on the formation and development of flower and fruit in *H. sabdariffa*.This result therefore confirmed further the two consecutive years' leachates of *T. diversifolia* and *C. odorata* as the optimal quantity of allelochemicals required for optimal production of *H. sabdariffa* plants since three years accumulated leachates remarkably suppress the formation and development of fruits in *H. sabdariffa*.

5. Conclusion

The study had shown that leachates of *T. diversifolia* (TWL) and *C. odorata* (CWL) under natural condition can enhance the seedling, vegetative, flowering and fruiting stages as well as the physiological (chlorophyll), and biomolecules (crude protein and ascorbic acid) contents of *H. sabdariffa* plant. Optimum enhancement of all these parameters however required two years' accumulated leachates plus exudates of *T. diversifolia* or *C. odorata*. The phytotoxic chemicals in the leachates plus exudates became accumulated or desorbed from the soil and affected negatively the production of *H. sabdariffa* plant after two years. The study therefore recommends two-year accumulated leachates of either weed as a potential crop–growth-enhancer whereas >2 years' leachates can be phytotoxic and detrimental to the crop's growth and yield. It also suggested the accumulation of ascorbic acid as possible adaptive mechanisms evolved by *H. sabdariffa* plants to allelopathic tolerance especially during juvenile stage. Highly important is the screening for the active ingredients in these weeds leachates for the production of crop-growth enhancer or regulator.

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