Interaction of Aqueous Extracts of Tithonia diversifolia, Chromolaena odorata and Kinetin Induced Growth and Accumulation of Chlorophyll in Hibiscus sabdariffa L.

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Abstract – A pot experiment was conducted under natural conditions to evaluate the effects of 15 ppm kinetin, aqueous extract of Tithonia diversifolia (FSET), Chromolaena odorata (FSEC), FSET-kinetin (TKN) and FSEC-kinetin (CKN) interaction on the vegetative growth and chlorophyll accumulation in Hibiscus sabdariffa. Results showed that application of FSET and FSEC significantly increased the shoot height, stem girth, number of leaves, leaf area, leaf area ratio, shoot fresh and dry weights, chlorophyll b and total chlorophyll accumulations in H. sabdariffa plants while the root length, fresh and dry weights were significantly reduced. This indicates that allelochemicals in these extracts can be successfully exploited for enhancing H. sabdariffa productivity. Kinetin significantly enhanced all the studied growth parameters except the leaf area ratio and chlorophyll a. This reveals the ameliorative potential of kinetin on the inhibited root system of allelopathic-soil grown plants. The data also showed that the FSEC-growth promoting potential nearly equal that of kinetin, thus suggesting the use of FSEC instead of expensive kinetin by the poor, small scale farmers for enhanced production of this crop. The interaction between aqueous extracts and kinetin (TKN and CKN) however, geometrically promoted the growth and chlorophyll accumulation in H. sabdariffa plants than the extracts or kinetin alone. The increase was more pronounced in the CKN than the TKN. This shows that kinetin can potentially synergize with the aqueous extracts to boost the production of this crop and recommends CKN for optimal growth and production of H. sabdariffa plants.

Keywords – Allelochemicals, Chlorophyll, Chromolaena Odorata, Hibiscus Sabdariffa, Tithonia Diversifolia.

I. INTRODUCTION

Justification for this study is found in the high cost as well as ecologically-unfriendly effects of long and short term use of inorganic fertilizer in Nigeria. This has resulted into diligent searching for cheaper and ecologically-friendly alternatives by small-scale resource poor farmers. Unfortunate enough, these they did usually with little or no scientific data and management strategies. There is dearth of information on the use of leachates of different weeds alone or in combination with hormone for stimulating the final yield of agricultural crops. This study therefore reveals the potential of the aqueous extract of Chromolaena odorata and Tithonia diversifolia separately and in combination with kinetin hormone to enhance the growth and yield of H. sabdariffa in Nigeria.

Tithonia diversifolia (Hemsl) A. Gray and Chromolaena odorata L. King and Robinson, commonly known as Mexican sunflower and Siam weed respectively are popular, perennial weedy asteraceous shrubs in Southwest Nigeria. Both weeds have dominated almost all fallowed land in this region [Ademiluyi, 2012]. T. diversifolia and C. odorata are reported to be highly allelopathic and are comparable with some of the other weeds that are ranked worst in the world [Ambika and Poornima, 2004; Otusanya et al., 2007]. However, at a particular concentration, C. odorata allelochemicals increase the vegetative growth, metabolite contents and yield in pulses, cereals and vegetables [Ambika and Poornima, 2004]. These weeds contain water-soluble allelochemicals in quantities ample enough to influence the growth and development, chlorophyll and biochemical accumulation, distribution and behaviour of neighbouring plants [Taiwo and Makinde, 2005; Otusanya et al., 2007; Otusanya and Ilori, 2012; Adetayo et al., 2005]. Ikewuchi et al., (2013) reported that C. odorata extracts contain compounds like stigmasterol/brassinosteroids which are growth regulators and signaling molecules essential for normal plant growth [Rao et al., 2002; Ayad et al., 2009] as well as conferring resistance to plants against various abiotic stresses [Priti, 2003]. Phenolics such as p-hydroxybenzoic acid and p-coumaric acids found in C. odorata had also been implicated in the protein synthesis through incorporation of amino acid (35 S-methionine) in crops seedlings [Baziramakenga et al., 1997; Inderjit and Nayyar, 2002]. Similarly, compounds such as phenols, alkaloids, terpenoids, sesquiterpenes lactones, tagitinin A and C, tannic acid, flavonoids and glycosides have been detected in the aqueous extracts of T. diversifolia [Taiwo and Maikinde, 2005; Otusanya and Ilori, 2012]. These compounds are potential plant growth promoters and their phytotoxicity (in case of higher concentrations) are reduced or completely degraded through soil microbes especially in the tropical region [Tian et al., 1992; Taiwo and Makinde, 2005; Aladejimokun et al., 2014]. One of the most commonest, abundant and important allelochemicals in both weeds aqueous extracts is phenolic acids [Ikewuchi et al., 2013; Fasola and Iyamah, 2014]. These compounds have widely been reported to stimulate the plant growth and functions [Hegab et al., 2008; Ghaereib et al., 2010]. Several investigators showed that phenolic acids enhanced the crops growth by mobilizing metabolites like carbohydrate and proteins and also through protection against environmental and biological stress such as high energy radiation exposure, bacterial infection or fungal attacks, cold stress and oxidative stress [Dillard and German, 2000; Tuzen and Ozdemir, 2003; Yoshioka et al., 2004; Adyanthaya 2007]. A number of sesquiterpene lactones are also known to possess this property [Fischer et al., 1989; Chen and Leather, 1990].

Similar to the growth promoting substances in aqueous extracts of T. diversifolia and C. odorata, hormones
produced by plants are known to play significant roles in promoting plant growth [Morgan, 1979; Nickel 1982; Harms and Opfinger, 1993], hence they are called plant growth promoting regulators (PGRs). PGRs have substantial growth stimulatory effects at lower concentrations [Harms and Opfinger, 1993] and beyond a certain range have inhibitory effects also [Gamaler and Glick, 2011]. Several researchers had shown the stimulatory effects of PGRs on germination of seeds, seedling and vegetative growth and yield of vegetables in the presence or absence of allelochemicals [Rai et al., 1986; Illiev et al., 2001; Terzi and Kocacaliskan 2009].

Cytokinins are very important PGRs used for stimulating cell division, as well as for the formation and growth of axillary buds and shoots. This group of PGRs consists of naturally occurring compounds such as zeatin, zosp, kinetin, and synthetic benzyladenine. Abdel-Rahman and Abdel-Aziz [1983] recorded an increased vegetative growth in *Datura innoxia* L. plant sprayed with different kinetin concentrations. El-Kettawi and Croteau [1987] later found that spraying of kinetin or benzyladenine on the leaves of sage and mint plants could significantly increased the number of leaves on the plants. Likewise, increase in the number of leaves on sage and *H. sabdariffa* plants was obtained by Mazrou et al., [1988] and Eraki [1994] following foliar application of benzyladenine (synthetic cytokinin) and ethrel respectively. Moreover, kinetin stimulated the synthesis of polysaccharides and the materials of the new cell walls in kinetin- treated *Phaseolus vulgaris* plants [Robertson et al., 1999] which resulted in the increase in shoot and root weights of the plant. Azza et al., [2011] indicated that 40 ppm and 20 ppm kinetin respectively increased the shoot height of *croton* (*Coededium variegatum*) by 50% and 33.3%. However, the production and activities of plant hormones are usually regulated by allelochemicals at low concentration usually through acting as promoting agent for the process of cell division and cell enlargement and thus tissue formation [Farooq et al., 2013]. Some allelochemicals inhibit IAA-oxidase which is known for hindering cell enlargement and plant growth by inactivating IAA. In this way, allelochemicals affect the role of a major plant hormone and resultantly improves plant growth [Rice, 1984].

*Hibiscus sabdariffa* L. var. *sabdariffa* (Malvaceae) is a short day annual which is native to Africa and common especially, the savanna region of West and Central Africa [McClinstock and El-Tahir, 2004]. The calyx of the *H. sabdariffa* L. var. *sabdariffa* is used for the preparation of drink known as “Zobo” while the calyx of the *H. sabdariffa* L. var. *alissima* is used to cook soup. Medicinally and pharmacologically, leaves and calyces infusions of this plant are regarded as diuretic, cholericetic, hypotensive, antispasmodic, and antibacterial [Duke, 1985; Adegunloye et al., 1996; Haji and Haji, 1999]. It constitutes a very rich source of ascorbic acid which protects human from several ailments [Morton, 1987; Amin et al., 2008]. Based on the importance and increased demand for optimal production of *H. sabdariffa*, this study evaluated the effects of: kinetin, aqueous extracts of *T. diversifolia* and *C. odorata* separately and the interactive effects of kinetin with each extract on the growth and chlorophyll contents of *H. sabdariffa* plants.

II. MATERIALS AND METHODS

A. Experimental Site and Materials Collection

The experiment was carried out at the Department of Botany, Obafemi Awolowo University, Ile-Ife. The seeds of *H. sabdariffa* used were collected from National Horticultural Research Institute (NIHORT) Ibadan and seeds of *T. diversifolia* were collected along Ede road, near the Obafemi Awolowo University (O.A.U.) main campus gate. The young seedlings of *C. odorata* were collected along the road, beside the site for the Botany Department afforestation scheme, O.A.U. Ile-Ife.

B. Preparation of Aqueous extracts of *C. odorata* and *T. diversifolia*

Extraction procedures were carried out according to the method of reference [Ahn and Chung, 2000]. Briefly, 72g weighed shoot of each of *T. diversifolia* and *C. odorata* were cut separately into small chips of about 4cm length and finely ground in a wet Philips (HR 2815) kitchen blender. The blended material was soaked in 1 litre distilled water for 12 hours. The solution was first filtered through cheese cloth to remove the fibre debris and afterwards filtered through Whatman No. 1 filter paper. The filtrate obtained served as fresh shoot aqueous extract (FSE).

C. Soil Culture

Plastic pots (5 litre capacity with a depth of 75cm) were filled with top soil. Each pot has six holes perforated at the bottom for good drainage. 10 sterilized seeds of *H. sabdariffa* were sown in each pot and supplied with 600 ml tap water every morning for two weeks. Two weeks after planting, the seedlings in each pot were thinned down to 6 per pot. Thereafter, these pots were randomly allocated to six treatment groups with the subscripts as follows; (i) CONTROL (supplied with distilled water only), (ii) FSEC (supplied with fresh shoot aqueous extract of *T. diversifolia* (iii) FSEC (supplied with fresh shoot aqueous extract of *C. odorata*), (iv) TKN (supplied with FSEC and sprayed with 15ppm kinetin solution), (v) CKN (supplied with FSEC and sprayed with 15ppm kinetin solution), (vi) KN (supplied with distilled water and sprayed with 15ppm kinetin solution). The pots in the control and extract-treated groups were respectively supplied with 600 ml of distilled water and appropriate aqueous extract on a daily basis while foliar application of kinetin was carried out on weekly interval. The experiment was set out in a completely randomized block design.

D. Harvest

Plants were harvested just before treatment started. Thereafter, harvesting of the seedlings was on weekly interval for a period of six weeks. Growth parameters such as shoot height, stem girth, number of leaves and root length were measured using standard methods. Leaf area was determined using the formula according to reference [Pearcy et al., 1989] and the Leaf Area Ratio (LAR) was calculated. Five randomly selected plants from each
regime were carefully uprooted and separated into shoot and root. The root was washed in the water basin to remove the soil particles. The fresh weight of the shoots and the roots were determined on a Mettler Toledo (PB203) electronic balance after being washed and mopped. Each of the plant shoots and roots harvested for fresh weight was packaged and labeled separately in envelopes. These were oven-dried to constant weight at 80°C in a Gallenkamp oven (Model IH-150). Each packaged dried plant part was then weighed on a Mettler Toledo (PB153) balance to obtain the dry weight. Chlorophyll contents of the fresh shoot were extracted with 80% acetone and quantified following the formula of reference [Combs et al., 1985]. Chlorophyll 'a' (μM) = 13.19A_{664} - 2.37A_{647} Chlorophyll 'b' (μM) = 22.10A_{664} - 5.26A_{647} Total Chlorophyll (μM) = 7.93A_{664} + 19.53A_{647} A_{664} is the absorbance at 664 nm; A_{647} is the absorbance at 647 nm.

E. 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.

III. RESULTS

A. Growth Parameters:

The shoot height of *H. sabdariffa* plants was significantly increased by all the treatments at *p*<0.05 (Table 1). The highest and lowest plant heights were recorded for CKN (130.68 cm) and the control (53.68 cm) respectively. Application of aqueous extracts of *T. diversifolia* (FSET), *C. odorata* (FSEC), kinetin (KN) and FSET plus kinetin (TKN) induced respectively 78.8, 96.4, 3.52, and 6.25% enhancement of the number of leaves on the aqueous extracts- and extract plus kinetin treated plants enhanced the leaf area than those treated with aqueous extract of *T. diversifolia* plus kinetin (TKN). It could be deduced from this result that additional application of kinetin to extract-treated plants enlarged the leaf area than application of extracts alone. This result further confirmed that synergy between aqueous extracts of *C. odorata* and kinetin (CKN) can function in the growth and development of leaf.

Table I: Effect of different aqueous extracts separately and in combination with kinetin on the shoot height and Number of Leaves on *H. sabdariffa*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot Height (cm) + S.D</th>
<th>No of leaves + S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>53.68 ± 3.52</td>
<td>21.85 ± 2.93</td>
</tr>
<tr>
<td>CKN</td>
<td>130.60 ± 3.52**</td>
<td>74.83 ± 2.93**</td>
</tr>
<tr>
<td>TKN</td>
<td>119.60 ± 3.52**</td>
<td>67.25 ± 2.93**</td>
</tr>
<tr>
<td>KN</td>
<td>107.93 ± 3.52**</td>
<td>60.1 ± 2.93**</td>
</tr>
<tr>
<td>FSEC</td>
<td>105.40 ± 3.52**</td>
<td>53.28 ± 2.93**</td>
</tr>
<tr>
<td>FSET</td>
<td>96.00 ± 3.52**</td>
<td>47.00 ± 2.93**</td>
</tr>
</tbody>
</table>

** means significantly different from the control at *p*<0.05. S.D. means Standard Deviation

Table II: Effect of different aqueous extracts separately and in combination with kinetin on the Leaf Area and Leaf area Ratio of *H. sabdariffa*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Leaf Area (cm²) + S.D</th>
<th>Leaf Area Ratio + S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>113.60 ± 23.74</td>
<td>114.75 ± 6.25</td>
</tr>
<tr>
<td>CKN</td>
<td>519.48 ± 23.74**</td>
<td>64.94 ± 6.25**</td>
</tr>
<tr>
<td>TKN</td>
<td>446.98 ± 23.74**</td>
<td>64.31 ± 6.25**</td>
</tr>
<tr>
<td>KN</td>
<td>330.94 ± 23.74**</td>
<td>71.17 ± 6.25**</td>
</tr>
<tr>
<td>FSEC</td>
<td>356.22 ± 23.74**</td>
<td>88.39 ± 6.25</td>
</tr>
<tr>
<td>FSET</td>
<td>295.20 ± 23.74**</td>
<td>82.46 ± 6.25</td>
</tr>
</tbody>
</table>

** means significantly different from the control at *p*<0.05. S.D. means Standard Deviation

Table III: Effect of different aqueous extracts separately and in combination with kinetin on the Stem Girth and Root Length of *H. sabdariffa*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Stem Girth (cm) + S.D</th>
<th>Root Length (cm) + S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>2.21 ± 0.14</td>
<td>19.35 ± 1.69</td>
</tr>
<tr>
<td>CKN</td>
<td>5.15 ± 0.14**</td>
<td>36.88 ± 1.69**</td>
</tr>
<tr>
<td>TKN</td>
<td>4.83 ± 0.14**</td>
<td>17.70 ± 1.69</td>
</tr>
</tbody>
</table>
** ** means significantly different from the control at p<0.05, S.D. means Standard Deviation

** Table IV: Effect of different aqueous extracts separately and in combination with kinetin on the Shoot fresh and Dry Weights of H. sabdariffa **

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot Fresh Weight (g) ± S.D</th>
<th>Shoot Dry Weight (g) ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>10.93 ± 2.84</td>
<td>0.99 ± 0.29</td>
</tr>
<tr>
<td>CKN</td>
<td>79.63 ± 2.84**</td>
<td>8.00 ± 0.29**</td>
</tr>
<tr>
<td>TKN</td>
<td>70.00 ± 2.84**</td>
<td>6.93 ± 0.29**</td>
</tr>
<tr>
<td>KN</td>
<td>58.15 ± 2.84**</td>
<td>4.65 ± 0.29**</td>
</tr>
<tr>
<td>FSEC</td>
<td>48.58 ± 2.84**</td>
<td>4.03 ± 0.29**</td>
</tr>
<tr>
<td>FSET</td>
<td>34.73 ± 2.84**</td>
<td>3.58 ± 0.29**</td>
</tr>
</tbody>
</table>

** ** means significantly different from the control at p<0.05, S.D. means Standard Deviation

** Table V: Effect of different aqueous extracts separately and in combination with kinetin on the Root fresh and dry weights of H. sabdariffa **

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root Fresh Weight (g) ± S.D</th>
<th>Root Dry Weight (g) ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>1.80 ± 0.49</td>
<td>0.20 ± 0.05</td>
</tr>
<tr>
<td>CKN</td>
<td>3.42 ± 0.49**</td>
<td>0.40 ± 0.05**</td>
</tr>
<tr>
<td>FSEC</td>
<td>1.57 ± 0.49**</td>
<td>0.18 ± 0.05</td>
</tr>
<tr>
<td>FSET</td>
<td>0.76 ± 0.49**</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>KN</td>
<td>3.77 ± 0.49**</td>
<td>0.44 ± 0.05**</td>
</tr>
<tr>
<td>TKN</td>
<td>3.07 ± 0.49**</td>
<td>0.33 ± 0.05**</td>
</tr>
</tbody>
</table>

** ** means significantly different from the control at p<0.05, S.D. means Standard Deviation

** Table VI: Effect of different aqueous extracts separately and in combination with kinetin on the Chlorophyll accumulation of H. sabdariffa **

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Chlorophyll a (µm) ± S.D</th>
<th>Chlorophyll b (µm) ± S.D</th>
<th>Total Chlorophyll (µm) ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTR</td>
<td>4.42 ± 0.54</td>
<td>1.22 ± 0.60</td>
<td>5.64 ± 0.67</td>
</tr>
<tr>
<td>CKN</td>
<td>5.89 ± 0.54**</td>
<td>4.05 ± 0.60**</td>
<td>9.95 ± 0.67**</td>
</tr>
<tr>
<td>FSEC</td>
<td>6.27 ± 0.54**</td>
<td>3.11 ± 0.60**</td>
<td>9.38 ± 0.67**</td>
</tr>
<tr>
<td>FSET</td>
<td>5.11 ± 0.54</td>
<td>4.07 ± 0.60</td>
<td>9.34 ± 0.67**</td>
</tr>
<tr>
<td>KN</td>
<td>5.66 ± 0.54</td>
<td>3.73 ± 0.60</td>
<td>9.40 ± 0.67**</td>
</tr>
<tr>
<td>TKN</td>
<td>5.16 ± 0.54</td>
<td>4.56 ± 0.60</td>
<td>9.76 ± 0.67**</td>
</tr>
</tbody>
</table>

** ** means significantly different from the control at p<0.05, S.D. means Standard Deviation

Variations in the leaf area ratio (LAR) of H. sabdariffa plant as induced by the application of FSET, FSEC, KN, TKN and CKN are shown in Table 2 above. The data showed that the control and TKN-treated plants had the highest (114.75) and the lowest (64.31) LAR respectively. Application of FSET and FSEC reduced the LAR of H. sabdariffa plants by 28.14 and 22.97% respectively. Contrary to the increasing trend observed for the number of leaves and leaf area, the LAR of H. sabdariffa plants were significantly reduced by the application of KN, TKN and CKN at P<0.05. The reduction effects followed the order CONTROL> FSEC>FSET>KN>CKN>TKN.

The effects of the FSET, FSEC, KN, TKN and CKN on stem girth, shoot fresh and dry weights of H. sabdariffa are shown in Table 3&4. The data showed that application of FSEC as well as FSET alone significantly enhanced the stem girth, the shoot fresh and dry weights. However, additional spraying of kinetin on these plants induced pronounced enhancements. This result emphasized again the greater potential of aqueous extracts plus kinetin to boost the production of H. sabdariffa than either extracts or kinetin alone.

The variations induced by the five treatments on H. sabdariffa root length, fresh weight and biomass were shown in Table 3&5. Unlike the shoot fresh and dry weights, the root lengths, fresh and dry weights of H. sabdariffa plants were slightly reduced by the application of aqueous extracts of T. diversifolia and C. odorata. While FSET reduced the root length, root fresh weight and biomass by 27.86, 57.78 and 55% respectively, the FSEC caused 8.53, 12.78 and 10% decrease in these parameters. Interestingly, additional spraying of kinetin on plants in the CKN and TKN regimes caused a significant elongation as well as deposition of materials in the roots of these plants. Compare with the control, the CKN plants recorded 90.59, 90 and 100% boost in the root length, root fresh and dry weights while 76.95, 70.56 and 65% increase were obtained for the TKN plants. However, the effects of these treatments followed the order; KN>CKN>TKN >CONTROL> FSEC>FSET. The practical implication here is that FSET is more phytotoxic to the root growth of H. sabdariffa plant than FSEC and that kinetin can potentially ameliorate or alleviate such inhibition.

** B. Photosynthetic Pigments Accumulation **

The chlorophyll contents in both the treated and the control H. sabdariffa plants are as shown in Table 6. The control plants had the lowest contents of chlorophyll a, b and total chlorophyll throughout the experimental period. The accumulation of chlorophyll a was significantly increased by the application of both CKN and FSEC while application of KN, TKN and FSET resulted in a slight stimulation of this chlorophyll. This result showed the greater tendency of aqueous extracts of C. odorata to enhance the formation and accumulation of chlorophyll a than the aqueous extracts of T. diversifolia. It also showed that application of KN alone could not significantly enhance chlorophyll a accumulation but in synergy with FSEC, it could. In the case of chlorophyll b, the highest stimulation of 273.77% was recorded for the TKN-treated plants, while application of FSET and FSEC accounted for 233.61 and 155.16% increase compared with the control. Application of KN enhanced the chlorophyll b contents by 205.74. Similarly, application of KN, FSET and FSEC stimulated significantly the total chlorophyll accumulation of the test crop at p<0.05. However, pronounced enhancement were obtained by combining the aqueous extracts and kinetin at P<0.05. Statistically, the plants in the treated regimes recorded higher chlorophyll b and total chlorophyll accumulation than the control plants at P<0.05.
IV. DISCUSSION

A wide array of allelochemicals is released into the environment in appreciable quantities via volatilization and exudation as leachates through the rain-wash of leaves and during their decomposition. These are known to play major roles in the growth and development of several crops [Liu and Lovette, 1993]. In this study, the aqueous extracts of T. diversifolia and C. odorata significantly stimulated the growth of H. sabdariffa in agreement with the results reported by Adetayo et al., (2005), Oyerinde et al., (2009) and Aladejimokun et al., (2014) where the leaf extracts of T. diversifolia and C. odorata separately promoted the growth of maize (Zea mays), cowpea (Vigna unguiculata), and tridax (Tridax procumbens). The results also showed that the allelochemicals from T. diversifolia and C. odorata can be successfully exploited for enhancing H. sabdariffa production. In other words, stimulation of the growth by these aqueous extracts suggested that phenolic acids, sesquiterpene lactones and flavonoids were not only abundant but were probably the stimulatory functions in the extracts. Some investigators believe that phenolic acids function as plant growth promoters since the compounds reportedly stimulate Indole acetic acid (IAA), gibberellic acid (GA₃) and kinetin activity in plants [Mukharjee and Kumar, 2007; Buer et al., 2010]. Phenolics also increase IAA oxidase, polyphenol oxidase, isoperoxidase and catalase activities [Singh et al., 2013], mobilize carbohydrates and proteins [Towers and Abysekerer, 1984] regulate photoperiodism and floral induction [Ebrahimzadeh and Abirashmchi, 2001]. Also, its compounds had been implicated in reducing denitrification through inhibition of nitrate reductase activity, thereby enhancing the conservation of nitrogen in the soil [Rui-xia, 2000]. This author added that three phenolic acids (trans-ferulic acids, benzoic acid and p-hydroxybenzoic acid) could synergistically adjust and stabilize the soil pH at the range of 7-8, so as to conserve soil nitrogen or reduce denitrification. All these are characteristics already implicated in the enhancement and/or triggering of plant growth and yield. Similarly, flavonoids had been implicated in inhibiting the generation of reactive oxygen species and then quench ROS once they are formed [Agati et al., 2012] while a number of sesquiterpene lactones are known to possess plant growth promoting property comparable enough to the known PGRs which are otherwise expensive [Fischer et al., 1989; Chen and Leather, 1990; Batish et al., 1996]. However, this enhancement could have been indirectly resulted from inactivation/ detoxification of the phytotoxic compounds in the aqueous extracts by the soil microbes. According to reference [Taiwo and Makinde, 1987], the allelochemicals in these aqueous extracts are biodegradable, therefore, can be added to the soil through microbial activities on the aqueous extracts. Tian et al., [1992] had earlier reported that the biologically inhibitive role of polyphenols does not persist under humid tropical field conditions, due to leaching and decomposition of polyphenols. This according to Hodge et al., [2000] subsequently increases the soil nutrient status for optimal growth of crops. In fact, Bertin et al., [2003] affirmed that allelochemicals in the soil solution do not just regulate the bioavailability of organic and inorganic compounds in the environment, but also facilitate their absorption and transport from the soil. These probably explain the observed growth increase in the FSET and FSEC regimes. However, the growth of the plants treated with FSEC appeared more luxuriant and healthier than those in the regime treated with FSET. This showed that FSEC contains more stimulatory functions than the FSET, a situation that corroborates the finding of Aladejimokun et al., [2014] and Otusanya et al., [2015].

In the case of kinetin, the plants sprayed with kinetin alone recorded greater values for shoot height, stem girth, leaf area, number of leaves, shoot fresh and dry weights than those treated with FSEC alone. However, the differences in their values were not significant at P<0.05 for these parameters. In practice, the implication here is that application of FSEC, instead of expensive kinetin, can be adopted by the poor, small scale farmers (growing this crop) since the former is cost effective and environmentally-friendly than the latter. In fact, Ambika and Poornima [2004] reported that allelochemicals in the aqueous extracts of C. odorata could be used as liquid fertilizer for increasing the crop growth and yield. Earlier, Batish et al., [1996] emphasized the effectiveness of the plant growth stimulating compounds in plant extracts (sesquiterpene lactones) as being comparable to those of the known PGRs. The rapid elongation of the shoot, wider stem girth and increased in the number of leaves on kinetin-treated H. sabdariffa plants were not surprising. This may be explained on the basis that, kinetin can potentially stimulate the synthesis of auxin or act as an inhibitor of IAA- oxidase [Eimest, 1977; Saleh and Hemerg, 1980]. Mukhtar [2000] and Rawia et al., [2010] also stressed the ability of kinetin to stimulate cell division, vascular strand development, xylem differentiation, and vascular growth as well as enhance formation and growth of axillary buds and shoots. In another experiments, Shudo [1994] and Fischer et al., [1989] found that kinetin played roles in the enhancement of the water status of plant, leaf expansion, growth of lateral buds, shoot growth, nutrient mobilization and reduction of membrane injury by dehydration. All these roles of kinetin probably accounted for the statistically significant increase in the leaf area, number of leaves, shoot fresh and dry weights of H. sabdariffa plants observed in this regime. Similar results were obtained by Eraki [1994] on the shoot height of H. sabdariffa subjected to benzyladenine (synthetic cytokinin) treatment.

Reports on the interactions between PGRs and allelochemicals have been varied. While some observed positive synergy between these compounds [Tomaszewski and Thimmann, 1966; Taty and Sharma, 1985; Kathiresan et al., 1990], others reported no interaction at all [Shuab et al., 2013]. In the present study, the combination of the aqueous extracts of T. diversifolia or C. odorata with kinetin significantly enhanced the growth of H. sabdariffa plant than single application of kinetin or aqueous extracts.
alone. As earlier discussed, the two compounds (aqueous extracts and kinetin) are capable of triggering the growth of *H. sabdariffa* plant independently; therefore, the pronounced increase in the growth of the CKN and TKN-treated plants could be attributed to the synergy or additive reaction between these compounds. Similar result was reported by Hemberg, [1951], Tomaszewski [1961], Tomaszewski [1964], Tayal and Sharma, [1985] and Kathiresan et al., [1990].

Of all the studied growth parameters, only the root length, root fresh and dry weights of extracts-treated *H. sabdariffa* plants were found to be significantly inhibited (Table 3&5). Interestingly, these parameters were significantly enhanced by the application of kinetin alone (KN) and in combination with the extracts (CKN and TKN). The significant inhibition of the root length observed for the extract treated plants may be attributed to the continued accumulation of allelochemicals in the soil where the roots were growing or direct contact of the roots with growth inhibitory substances in the applied extract [Sangakkara et al., 2004; Ilori et al., 2007]. However, it could be species dependent as reported by Hedge and Miller [1990]. These authors found that the root system of many tested crops was more sensitive to phytotoxic compounds than their shoot system. Ilori et al., [2007] also observed significant reduction in the root length, root fresh and dry weight of *Oryza sativa* treated with aqueous extracts from different parts of *T. diversifolia*. Kinetin like other cytokinin is known for increasing the water and nutrient absorption by the root through stimulation of the xylem differentiation and vascular strand development. This probably explains the greatest root fresh and dry weight recorded by kinetin-sprayed-*H. sabdariffa* plants. Its role as stimulator of cell division and elongation in the root apical meristems and cambium explains the pronounced increase in the root length observed for the KN-treated plants over the control and other treatments. In the case of interaction of kinetin and aqueous extracts, a close relationship appeared to exist between the changes in the root growth and the endogenous levels of growth hormones. The root is the site of cytokinin biosynthesis in higher plants and transported through the xylem to the aerial portion of the plant [Azza et al., 2011]. It is suspected that the toxins in the allelochemicals after being absorbed at the root zone elicit their effect at the sensitive site, thus, triggering the internal contents of *H. sabdariffa* plant growth regulators such as decreasing its cytokinins, auxins and gibberellins level in the root. It could therefore be said that the root of *H. sabdariffa* plant was quite sensitive to the absorbed allelochemicals to the extent that synthesized cytokinins in the root of this plant could not alone overcome the inhibitory activity of these allelochemicals until exogenous kinetin was applied. Hale and Orcutt [1987] and Kabar, [1990] indicated that exogenously applied PGRs such as IAA and kinetin can overcome some stresses posed by drought, salinity and allelochemicals in plants. Kathiresan et al., [1990] observed the synergistic interactions between phenols and IAA and found that phenol-IAA synergism was well pronounced in root initiation and/or root elongation. The interaction of kinetin with aqueous extracts therefore seems to play certain roles in some phases of cell division, differentiation, elongation or mobilization of water and nutrients as well as deposition of materials in the root of *H. sabdariffa* plant which eventually resulted in the pronounced enhancement of these plants root parameters. Also, the exogenously applied kinetin might have supplied additional quantities of hormones or their precursors which were involved in the recovery of root growth of the test plant from the inhibition induced by FSET and FSEC. This recovery may however be a consequence of several roles played by such hormones, which can trigger the internal cellular metabolism and also induce alterations in the ratios of the growth regulators. These results corroborate the findings of Terzi and Kocacaliskan [2009] who reported that application of kinetin alleviated the juglone (allelochemical) stress, thereby increased the length, shoot and root fresh and dry weights of the seedlings. Basu et al., [1969] had earlier reported that phenolics compounds like salicylic acid, gallic acid and tannic acid when used alone did not show any conspicuous root promoting effect but did so with PGRs (IAA) in the case of leafy cuttings of *Eranthemum tricolorum*. Similar results were obtained by some earlier workers on *Croton variegatum*, *Casia officinalis*, *H. sabdariffa*, *Polianthus tuberosa* and ornamental plants [El-Sayed et al., 1989; Menesi et al., 1991; Mazrou et al., 1988; Eraki, 1994].

The finding that extracts-treated *H. sabdariffa* plants had higher contents of chlorophyll pigment than the control is similar to the study of Otusanya et al., [2008, 2014] who observed significant increase in the chlorophyll b and total chlorophyll of *Lycopersicum esculentum* and *Amaranthus dubius* treated with root exudates of *T. diversifolia*. It also corroborates the findings of Oyerinde et al., [2009]. The authors reported that aqueous extracts of *T. diversifolia* significant increase the chlorophyll b and total chlorophyll of *Ze a mays* plants. Gamalero and Glick [2011] stated that allelochemicals have positive role to play in chlorophyll accumulation, photosynthesis, and genetic encodings. The present findings therefore suggest that both chlorophyll synthesizing system and chlorophyllase activity might have been affected by the aqueous extracts probably through enhancement of the former and inhibition of the latter. Again, it could be due to the shielding of the photosynthetic systems by the phenolics in the applied extracts. According to Yoshioka et al., [2004], phenolic acids enhanced the growth of crops through protecting the plants against high energy radiation exposure. These actions could not have been one allelochemical effects, but rather synergistic interactions among these compounds. Williamson [1990] and Einhellig and Rasmussen [1978] stated that allelopathy are often due to synergistic activity of allelochemicals rather than to single compounds and under field conditions, this effect become significant even at low concentrations. However, the protective role of allelochemicals against plant pathogen which could have created optimal conditions for the growth and chlorophyll biosynthesis in extract-treated *H. sabdariffa* plants can not be overemphasized. All these factors and in collaboration with soil microbes could
probably account for the enhancement of the chlorophyll accumulation. The stimulatory effect of kinetin on pigment biosynthesis might be presumably due to the fact that, kinetin can increase the rate of transpiration [Haroun et al., 2003] and this might have increased the rate of translocation of minerals and root-synthesized cytokinins from the root to the developing shoot. Uheda and Kuraishi [1978] found that, kinetin increased simultaneously the transpiration and chlorophyll synthesis in etiolated squash cotyledons. Moreover, it could have been that the hormone exerted its effect on photosynthetic machinery at the mesophyll and chloroplast level by increasing plastids biogenesis and consequently increases the number of proplastids or newly developed chloroplasts in accordance with the findings of Aldequay and Baka [1998]. Other workers had demonstrated that kinetin can potentially enhance the maturation of chloroplasts, retain chlorophyll contents, prevent chlorophyll loss etc. in tobacco, sorghum and Xanthium leaves respectively [Stetler and Laetsch, 1965; Richmond and Lang, 1957; Alsokari, 2009]. The stimulatory effect of kinetin on the chlorophyll accumulation substantiates the finding of Sugiuara [1963] who observed significant increase in the chlorophyll synthesis of detached primary leaves treated with kinetin. Increase in the level of chlorophyll b in the shoot of H. sabdariffa plants treated with benzyladenine was reported by Zayed et al., [1985]. Iman and Youseff [1998] observed that benzyladenine increases chlorophyll a and b in H. sabdariffa plant. In the search for compounds which could stabilize the chlorophyll synthesis or ameliorate the pigments degradation reportedly induced by allelochemicals, kinetin hormone seemed appropriate to the researchers because of its effectiveness to stabilize leaf membranes, chlorophyll, soluble sugars and proteins in Ricinus communis plants growing in waterlogged soil [Fischer et al., 1989]. This hypothesis seems correct because of the pronounced chlorophyll accumulation recorded in the extracts plus kinetin treated H. sabdariffa plants compared with the plants in the control as well as those in KN, FSET and FSEC regimes. This showed that kinetin can potentially synergize with the extracts of T. diversifolia or C. odorata. The soil pH adjustment potential of the phenols in the aqueous extracts [Rui-xia, 2000] coupled with the ability of kinetin hormone to biosynthesize plastids, enhance chloroplasts maturation, retain chlorophyll or prevent chlorophyll loss [Stetler and Laetsch, 1965; Richmond and Lang, 1957; Alsokari, 2009] could probably account for these observations. Finally, this result can be likened to the finding of Salama and Awadalla [1987] and Gad-Allah and El-Enany [1999] who reported enhanced chlorophyll a and b contents in H. sabdariffa and Lupinus termis plants sprayed with kinetin and grown under cadmium or acid stress conditions.

V. CONCLUSION

The results of this study indicated that the allelochemicals from T. diversifolia and C. odorata can be successfully exploited for enhancing crop productivity. The latter contains allelochemicals whose growth promoting function is comparable with that of kinetin and therefore suggested the use of this extracts in place of expensive kinetin by the poor small scale farmers. The study also emphasized the ameliorative effects of kinetin on the root system of plants (H. sabdariffa) growing in allelopathic soil and the ability of the hormone to synergize with the aqueous extracts of T. diversifolia and C. odorata. The data presented here also showed that application of the duo of C. odorata extracts and kinetin increased the growth and chlorophyll contents of H. sabdariffa plants than the combination of aqueous extracts of T. diversifolia and kinetin and therefore suggested further studies on the screening for the active ingredients in both aqueous extracts. Lastly, application of aqueous extracts of C. odorata plus spray with adequate concentration of kinetin is recommended for achieving the survival, establishment and optimal growth and chlorophyll accumulation in H. sabdariffa plants.

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was born in Ilobu on the 1st of April 198x. Mr. Ogunwole attended Ilobu Secondary Commercial Grammar School, and later proceeded to Obafemi Awolowo University, Ile-Ife, Nigeria in 2001 to study Botany. He later enrolled for his Postgraduate Master degree in Botany (plant physiology option) and presently a Ph.D. student of the same university. He is presently a lecturer in the Department of Biological Sciences, Wesley University of Science and Technology, Ondo State Nigeria. His research interests include but not limited to: The search for alternatives to the use of inorganic fertilizers for improving crops yield. The weapons for achieving these aims focus majorly on the use of allelochemicals or aqueous extracts of weeds or crops, the use of hormone especially kinetin, the use of Boron (B) elements and combination of the two or all of these substances.

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