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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-hydroxylbenzoic 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 Maiknde, 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; Ghareib *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 Oplinger, 1993], hence they are called plant growth promoting regulators (PGRs). PGRs have substantial growth stimulatory effects at lower concentrations [Harms and Oplinger, 1993] and beyond a certain range have inhibitory effects also [Gamalero 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, zip, kinetin, kinetin riboside [Nickel 1982] 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-Keltawi 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 cytokining) 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 (*Coaedium* 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 [McClinctock 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. altissima* is used to cook soup. Medicinally and pharmacologically, leaves and calyces infusions of this plant are regarded as diuretic, cholerectic, 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 2815i) 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) FSET (supplied with fresh shoot aqueous extract of *T. diversifolia* (iii) FSEC (supplied with fresh shoot aqueous extract of *C. odorata*), (iv) TKN (supplied with FSET 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

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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 80^0C in a Gallenkhamp 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 formulae of reference [Combs' *et al.,* 1985].

Chlorophyll 'a' (μM) = $13.19A_{664} - 2.37A_{647}$

Chlorophyll 'b' (μM) = $22.10A_{647} - 5.26A_{664}$

Total Chlorophyll (μM) = $7.93A_{664} + 19.53A_{647}$

A_{664} is the absorbance at 664nm; A_{647} is the absorbance at 647nm.

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 (130.68cm) 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, 101.1 and 122.8% increase in the plant height compare with the control. However, there was no significant difference in the shoot height of KN and FSEC at p<0.05. The practical implication of this result is that soil application of FSET and FSEC, though could enhance the elongation of *H. sabdariffa* shoot, spraying the plants with kinetin would further enhance the height of the plant.

The data presented in Table 1 shows that application of FSEC, FSET, KN, CKN and TKN significantly enhanced the number of leaves on *H. sabdariffa* plants with the control and CKN-treated plants recording the lowest and highest number of leaves respectively. The number of leaves on the aqueous extracts-treated plants (FSEC and FSET) was lower than that of the kinetin and aqueous extract plus kinetin plants (CKN, TKN and KN). The enhancement of the number of leaves on *H. sabdariffa* plants followed the order; CKN>TKN>KN>FSEC> FSET>CONTROL. In other words, the number of leaves on the extract-treated plants was not significantly lower than that of the kinetin-treated plants at p<0.05. Of all the treatments, this result points to the interaction of kinetin and aqueous extracts (CKN) as the best inducer of leaf formation and growth in *H. sabdariffa* plant.

Table 2 showed that the leaf area of the control plants was 113.60 cm^2, while those of FSET, FSEC, KN, TKN and CKN-treated plants were 295.2, 356.22, 330.94, 446.98 and 519.48 cm^2 respectively representing 159.86, 213.57, 191.32, 293.47 and 357.29% increase over the control. Statistically, the leaf area of the control plants was significantly lower than that of the plants in the treated regimes. In other words, the leaf area of both the extract-treated and extract plus kinetin-treated plants were significantly greater than that of the control plants at p<0.05. Also, the enhancement induced by the application of extracts plus kinetin was more pronounced than those observed in the regime treated with extracts or kinetin alone. However, plants treated with CKN recorded greater number of leaves and 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 therefore 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*

Treatments	Shoot Height (cm)± S.D	No of leaves ± S.D
CTR	53.68 ± 3.52	21.85 ± 2.93
CKN	130.68 ± 3.52**	74.83 ± 2.93**
TKN	119.60 ± 3.52**	67.25 ± 2.93**
KN	107.93 ± 3.52**	60.1 ± 2.93**
FSEC	105.40 ± 3.52**	53.28 ± 2.93**
FSET	96.00 ± 3.52**	47.00 ± 2.93**

** 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

Treatments	Leaf Area (cm^2) ± S.D	Leaf Area Ratio ± S.D
CTR	113.60 ± 23.74	114.75 ± 6.25
CKN	519.48 ± 23.74**	64.94 ± 6.25**
TKN	446.98 ± 23.74**	64.31 ± 6.25**
KN	330.94 ± 23.74**	71.17 ± 6.25**
FSEC	356.22± 23.74**	88.39 ± 6.25
FSET	295.20± 23.74**	82.46 ± 6.25

** 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*

Treatments	Stem Girth (cm) ± S.D	Root Length (cm) ± S.D
CTR	2.21 ± 0.14	19.35 ± 1.69
CKN	5.15 ± 0.14**	36.88 ± 1.69**
TKN	4.83 ± 0.14**	17.70 ± 1.69

KN	4.58 ± 0.14**	13.96 ± 1.69
FSEC	4.4 ± 0.14**	40.95 ± 1.69**
FSET	3.93 ± 0.14**	34.24 ± 1.69**

** 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*

Treatments	**Shoot Fresh Weight (g) ± S.D**	**Shoot Dry Weight (g) ± S.D**
CTR	10.93 ± 2.84	0.99 ± 0.29
CKN	79.63 ± 2.84**	8.00 ± 0.29**
TKN	70.00 ± 2.84**	6.93 ± 0.29**
KN	58.15 ± 2.84**	4.65 ± 0.29**
FSEC	48.58 ± 2.84**	4.03 ± 0.29**
FSET	34.73 ± 2.84**	3.58 ± 0.29**

** 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*

Treatments	**Root Fresh Weight (g) ± S.D**	**Root Dry Weight (g) ± S.D**
CTR	1.80 ± 0.49	0.20 ± 0.05
CKN	3.42 ± 0.49**	0.40 ± 0.05**
FSEC	1.57 ± 0.49	0.18 ± 0.05
FSET	0.76 ± 0.49**	0.09 ± 0.05
KN	3.77 ± 0.49**	0.44 ± 0.05**
TKN	3.07 ± 0.49**	0.33 ± 0.05**

** 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*

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

** 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 thorughout 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* is 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 (GA_3) 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 Abeysekera, 1984] regulate photoperiodism and floral induction [Ebrahimzadeh and Abrishamchi, 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 [Einest, 1977; Saleh and Hemerg, 1980]. Mukhtar [20008] 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 Thimann, 1966; Tayal 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 *Eranthenum tricolor.* 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 *Zea 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 Aldesuquy 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 Sugiura [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 *Riccinus 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* plant) 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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