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Studies on the Allelopathic Effect of Aqueous Extracts of *Axonopus compressus* (Swartz) P. Beauv on Some Growth Parameters and Phenolic Content of *Kalanchoe pinnata* (Lam.) Pers.

By

[1]UZOMA. M. C, [1]MENSAH, S.I and *[1]OCHEKWU, E.B.
[1]Department of Plant Science and Biotechnology, University of Port Harcourt

Corresponding Author: Ochekwu, E.B. (edache.ochekwu@uniport.edu.ng)

Abstract

Plant-plant interactions could either be positive (stimulatory) or negative (inhibitory) to one or both plants involved, and the degree/level of positivity or negativity of the effect varies depending on the plants involved in the interaction. Studies were conducted to check the allelopathic effect of a neighbouring weed, *Axonopus compressus*, on the important medicinal plant, *Kalanchoe pinnata*, with special emphasis on the effect on its morphology, chlorophyll content, and phenolic content (which is the main active bioconstituent responsible for its medicinal properties). The experiment was carried out in a screenhouse by treating (watering) *Kalanchoe pinnata* every two days with 50ml of different concentrations (0%, 25%, 50%, 75%, 100% treatments) of 0.5g/ml stock solution of aqueous extracts of *Axonopus compressus*; and the parameters studied include shoot length, number of leaves, leaf area, root length, number of roots, fresh weight, dry weight, total chlorophyll content (TCC) and total phenolic content (TPC) of the treated *Kalanchoe pinnata*. Results from the experiment showed that, for all parameters studied, the treatments were significantly ($P \leq 0.05$) higher than the control. The morphological growth parameters, total chlorophylls content, and phenolic content of *K. pinnata* showed increased yields compared to the control, thereby establishing that aqueous extract of *A. compressus* showed a positive (stimulatory) allelopathic effect on *K. pinnata*.

Therefore, *A. compressus* could be used as phyto-fertilizer by incorporation into the soil in medicinal gardens, as this could enhance the morphological growth parameters and boost secondary metabolite production of *K. pinnata* considering its economic uses as an important medicinal plant in Nigeria and Africa at large.

Key words: Allelopathy, Aqeous extract, *Axonopus compressus*, Phenolic content, *Kalanchoe pinnata*

Introduction

Plant-plant interactions could either be positive (stimulatory) or negative (inhibitory) to one or both plants involved, and the degree/level of positivity or negativity of the effect varies depending on the plants involved in the interaction. The concept of plant-plant interactions is fully defined by two sub-concepts - Competition and Allelopathy. Competition defines the physical interaction between individuals (i.e. plants), brought about by a shared requirement for a particular resource in limited supply, and [often] leading to a reduction in the performance of, at least, some of the competing individuals (Weigelt and Jolliffe, 2003). Allelopathy defines the chemical interaction between plants in which one plant either inhibits or promotes the morphological and/or biochemical features of another plant by/through the release into the environment of certain biochemicals (called allelochemicals) which act either directly or is biotransformed to produce the desired effect. *Kalanchoe pinnata* (syn. *Bryophyllum pinnatum*), also recognized with the common names Miracle leaf, Air plant, Life plant or Goethe plant, is a succulent perennial plant that grows up to 3 – 5 feet tall. It has hollow stems, fleshy dark green leaves that are distinctly scalloped and trimmed in red, and bell-like pendulous flowers (Ghani, 2003). *Kalanchoe pinnata* is used as a horticultural plant, though this is rare in Nigeria where it is most widely cultivated as an important medicinal plant. *K. pinnata* is reported to exhibit antibacterial activity (Joseph *et al*., 2011; Okwu and Nnamdi 2011a, b), anti-cancer activity (Devbhuti *et al.,* 2012; Supratman *et al*., 2001), anti-parasitic activity (Muzitano *et al*., 2006), insecticidal activity (Supratman *et al.*, 2000), anti-allergic activity (Cruz *et al.*, 2012, 2008; Joseph *et al*., 2011), anti-inflammatory activity (Gupta *et al.*, 2010), anti-ulcer inducer properties (Lans, 2006), wound healing effect (Nayak *et al*., 2010; Yadav and Dixit, 2003), anti-diabetic activity (Goyal *et al.*, 2013), etc.

Other uses of *K. pinnata* in traditional medicine include its use as treatment for cough, earaches, eczema, inflammations and pimples (in Nigeria), eye infections, menstrual disorder and wounds (in Mexico), childbirth, cold, and respiratory infections (in Nicaragua), fever, constipation and piles (in Bangladesh), abscess, arthritis, athlete's foot, boils, burns, conjunctivitis, dermatitis, insect stings, intestinal problems, itch, kidney stones, mouth sores, rheumatism, toothache, ulcers, tumor and as a sedative (in Brazil), broken bones and bruises (in Ecuador), abdominal discomfort, diabetes,

diarrhea, dysentery, flatulence, indigestion, scabies, urinary insufficiency, swelling and insect bites (in India), chicken pox and stomachache (in USA), bacterial infections, migraine, nausea and cancer (in Peru). Other uses elsewhere include as treatment for earaches, malnutrition, paralysis, sprains, to cut umbilical cord in new born babies, and to expel worms (Quazi *et al*., 2011).

Most, if not all, of these ailments are typical Nigerian ailments. By virtue of the effectiveness of the traditional use of *Kalanchoe pinnata* to dispel these ailments, *K. pinnata* is an important medicinal plant in Nigeria and Africa at large, as it offers a cheap alternative to combating a plethora of typical Nigerian ailments suffered by the mostly poor natives. There has been both visual and literature evidence supporting the coexistence of *Kalanchoe pinnata* (an important medicinal plant) and *Axonopus compressus* (a weed) as they occur in the same habitat. *Axonopus compressus* (carpet grass) seems to be the major weed to the cultivated medicinal plant *Kalanchoe pinnata* (González de Léon *et al*, 2016; personal observations). Since weeds are reported to proliferate due to their allelopathic property (Pisula and Meiners, 2010), it is suspected that the weed (*Axonopus compressus*) may have an [allelopathic] effect on its important neighbour (*Kalanchoe pinnata*), especially on its medicinal properties as is contained in the amount of its phenolic content which is the main pharmacologically and biologically active phytoconstituent of *K. pinnata* (Rajsekhar *et al*., 2016; Anusha *et al*., 2014; Milad *et al*., 2014; Shazid *et al*., 2012; Quazi *et al*., 2011; Gaurav *et al*., 2007). This hypothesis therefore gives an objective to this study – to reveal how allelopathy of *Axonopus compressus* affects (either increases or decreases) the productivity/yield of *K. pinnata*, with special emphasis on its total phenolics.

Materials and Methods

Obtaining Sterilized Sand

River bed sand was sterilized by soaking in boiled water (100◦C) for 15 minutes, and then allowed to cool. This was done to remove external factors such as inherent seed banks and plant disease-causing agents. The pH level was determined using a pH meter (Suptra Scientific, Canada), and leveled up to the pH of 7.0±0.3 by adding drops of 1M NaOH.

Obtaining Aqueous Extract of *Axonopus compressus*

Whole plants of *Axonopus compressus* were collected around Abuja Campus, University of Port-Harcourt, Choba. The collected plants were rinsed in water to

remove dust and soil particles, and then air-dried for 30 minutes to remove surface water. The plants were pulverized using a manual grinder (Krish Industries, India); and 500g of the pulverized plant samples were macerated in 1000ml (1L) of distilled water for 72 hours (3 days) with intermittent agitation.

Obtaining Plantlets of *Kalanchoe pinnata*

Plantlets were obtained as clones from whole leaves of *K. pinnata* which were placed on a moist cotton towel, watered daily, and allowed to shoot. The plantlets, having grown roots, were detached from the leaves after two (2) weeks.

Experiment – Establishment of Allelopathic Relationship

Plastic pots (diameter = 14cm, depth = 6cm) were filled with 1kg of sterilized river bed sand. Two weeks old plantlets of *K. pinnata* (height = 0.3cm; number of leaves = 2) were planted at the three angles of an imaginary equilateral triangle (side = 6cm) in the sand-filled pots. The pots were watered daily at the centre of the equilateral triangle with 50ml *A. compressus* extract of 0%, 25%, 50%, 75% and 100% of stock solution. This experiment was run for 56 days (8 weeks), and the readings/datasets for the parameters were taken weekly while the root length, Total Phenolic Content (TPC) and Total Chlorophyll Content (TCC) were taken on the 8th week.

Experimental Design

The experiments were conducted adopting a Completely Randomized Design (CRD) with four replicates.

Parameters Under Study

Shoot length, Number of leaves, Leaf area, Root length, Number of roots, Fresh weight and dry weight of whole plant, Total Phenolic Content (TPC) and Total Chlorophyll Content (TCC)

Measuring the Shoot Length

The shoot length was measured using a ruler by measuring from the base of the plant to the topmost leaf. The shoot length was taken as the average of the shoot length of all the three plants in the pot.

Measuring the Number of Leaves

The number of leaves was obtained by visual counting of the leaves. The number of leaves was taken as the average of the number of leaves of all the three plants in the pot.

Measuring the Leaf Area

The leaf area was measured as the product of the leaf length and the leaf width of two biggest leaves. The leaf area was taken as

the average leaf area of the three plants in the pot.

Measuring the Root Length

The root length was measured using a ruler by measuring from the base of the root to the root tip. The root length for one plant was taken as the average of the two longest roots; while the root length for one pot was taken as the average root length of the three plants in a pot.

Measuring the Number of Roots

The number of roots was obtained by visual counting of the roots. The number of roots was taken as the average of the number of roots of all the three plants in a pot.

Measuring the Fresh Weight

The plants were weighed using a weighing balance. The average weight of the three plants in a pot were taken as the fresh wet weight.

Measuring the Dry Weight

The plants were oven-dried at 80°C for 72 hours. The individual dry weights of the plants were weighed using an electronic weighing balance; and the average of the three plants in a pot was taken as the dry weight.

Measuring the Total Phenolic Content (TPC) and Total Chlorophyll Content (TCC)

The oven-dried plant samples (0%, 25%, 50%, 75% and 100% treatments) were pulverized and tagged T_1, T_2, T_3, T_4 and T_5 respectively.

(a) Measuring the Total Phenolic Content (TPC)

The amount of total phenolics in the plant extract was determined spectrophotometrically following the method of Sunita and Dhananjay (2010) with some slight modifications. The total phenolic content in the pulverized plant samples was determined with the Folin-Ciocalteu reagent. Methanolic solutions of the pulverized plant samples (T_1, T_2, T_3, T_4 and T_5) were prepared in the concentration of 1 mg/ml for the analysis. The reaction mixture was prepared by introducing 0.5ml of each methanolic solution into test tubes, then mixed with 2.5ml of 10% Folin-Ciocalteu reagent and 2ml of 7.5% Sodium carbonate (Na_2CO_3). The test tubes were allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm spectrometrically. All determination was done in triplicate and the mean value obtained.

The same procedure was repeated for standard solution of Gallic acid (0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml of Gallic

acid in methanol); and the calibration curve was read (mg/ml), and the content of phenolics in the extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

(b) Measuring the Total Chlorophyll Content (TCC)

The total chlorophylls content (TCC) was determined using the method of Sumanta *et al*., (2014) with some modifications. 2g of pulverized plant sample was macerated in 10ml of Methanol for 15 minutes at room temperature (28ºC). The solution was filtered, and the solution mixture was analyzed for Chlorophyll-a, Chlorophyll-b contents using a spectrophotometer by measuring the absorbance at 652.4nm and 665.2nm. The equation used for the quantification of Chlorophyll-a, Chlorophyll-b and Total Chlorophyll Content (TCC) is shown below:

$Ch\text{-}a = 16.72A_{665.2} - 9.16A_{652.4,}$

$Ch\text{-}b = 34.09A_{652.4} - 15.28A_{665.2,}$

$TCC = Ch\text{-}a + Ch\text{-}b$

Data Analysis

All data collected were subjected to statistical analysis using SAS (2007 Version 9.1). The means were separated using Least Significant Difference (LSD) at 5% level of probability.

Results

Effect of Treatments on Shoot Length of *Kalanchoe pinnata*

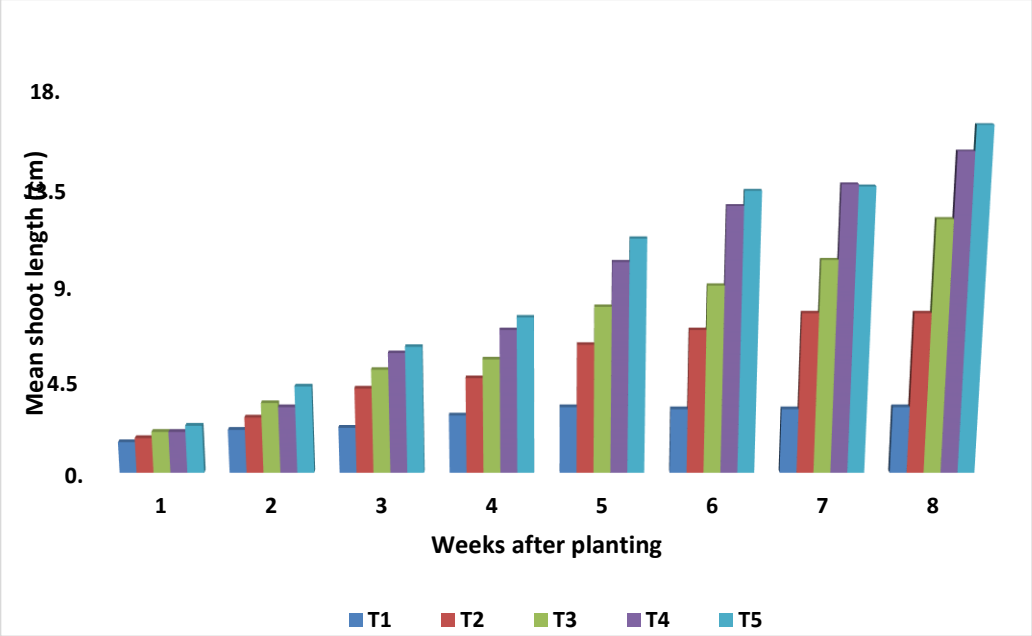

Fig 1 : Mean shoot length of *K. pinnata* at different treatments over 8 weeks after planting (WAP)

A linear increase in shoot length was observed with an increase in concentration of treatment. The highest shoot length was observed at week 8 for T5 (100% treatment) with a mean shoot length of 16.4±0.635cm; while the lowest shoot length was recorded at week 1 by T1 (control) with a mean shoot length of 1.5±0.287cm. At week 8, T5 (16.4±0.635cm) had a 500% increase in shoot length compared to the control, T1 (3.2±0.265). This shows 5 times increase in shoot length of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Fig 1).

Effect of treatments on number of leaves of *Kalanchoe pinnata*

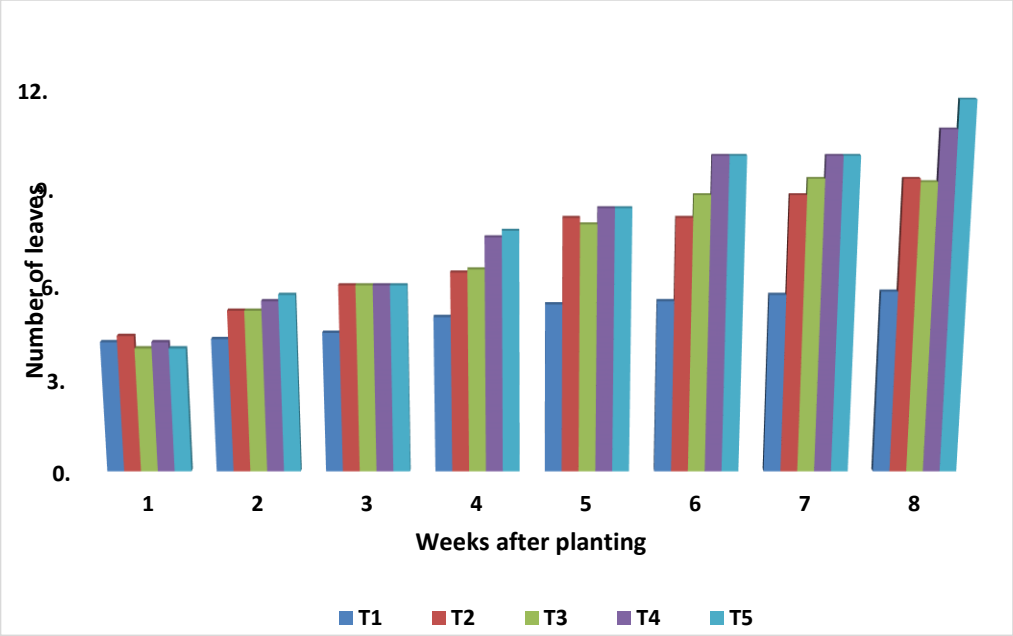

Fig 2: Mean number of leaves of *K. pinnata* at different treatments over 8 weeks after planting (WAP)

The number of leaves of *K. pinnata* showed a slight linear increase with increasing treatment. The highest number of leaves was observed at week 8 for all treaments. T5 (100%) with a mean number of leaves of 11.7±0.40 was the highest; while the lowest number of leaves was observed at week 1 for T3 (50% treatment) and T5 (100% treatment) with mean number of leaves of 4.0±0.0. At week 8, T5 had a 200% increase in number of leaves compared to the control T1. This showed a x 2 increase in number of leaves of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Figure 2).

Effect of Treatments on Leaf Area of *Kalanchoe pinnata*

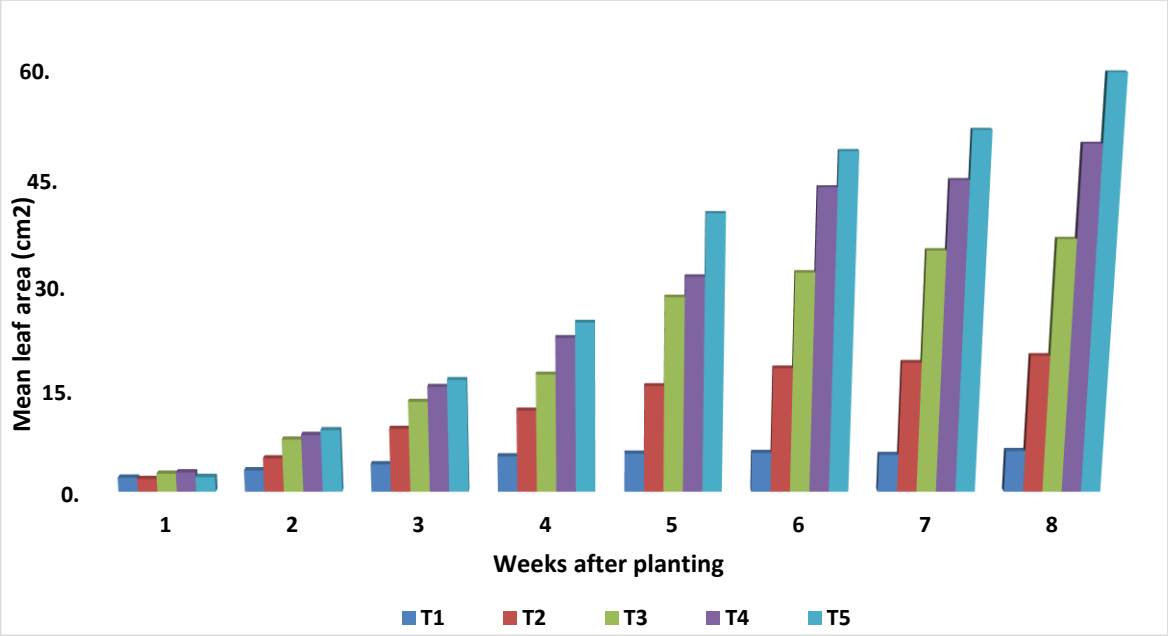

Fig 3: Mean leaf area of *K. pinnata* at different treatments over 8 weeks after planting (WAP)

The leaf area of *K. pinnata* showed a linear increase with increasing treatment. The highest leaf area was observed at week 8 for T5 (100% treatment) with a mean leaf area of 59.6±3.984cm^2; while the lowest leaf area was observed at week 1 for T2 (25% treatment) with a mean leaf area of 1.9±0.216cm^2. At week 8, T5 had approximately 1000% increase in leaf area compared to the control, T1. This shows a 10 x increase in leaf area of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Fig. 3).

Effect of treatments on fresh weight of *Kalanchoe pinnata*

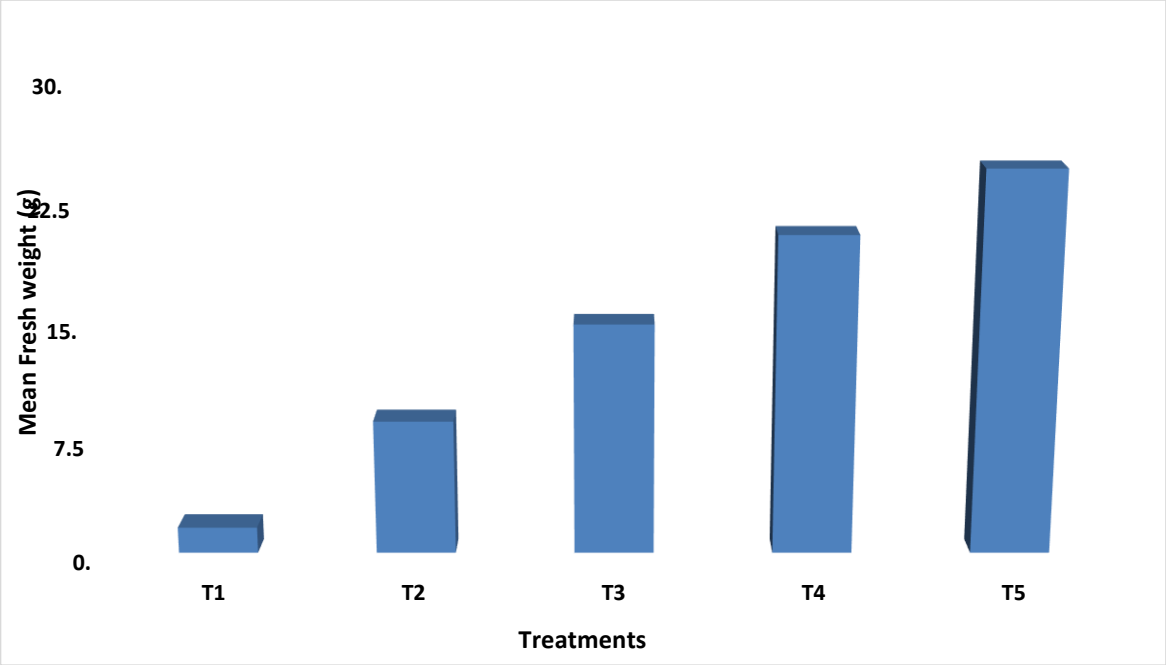

Fig. 4: Mean fresh weight of *K. pinnata* at 8 weeks after planting

A linear increase in leaf area was observed with an increase in concentration of treatment. The highest fresh weight was observed for T5 (100% treatment) with a mean wet weight of 24.65±1.334g while the lowest was observed for the control (T1) with a mean wet weight of 1.69±0.514g. T5 had an approximate 1400 % increase in wet weight compared to the control, T1. This showed a 14 x increase in wet weight of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Fig 4).

Effect of treatments on root length of *Kalanchoe pinnata*

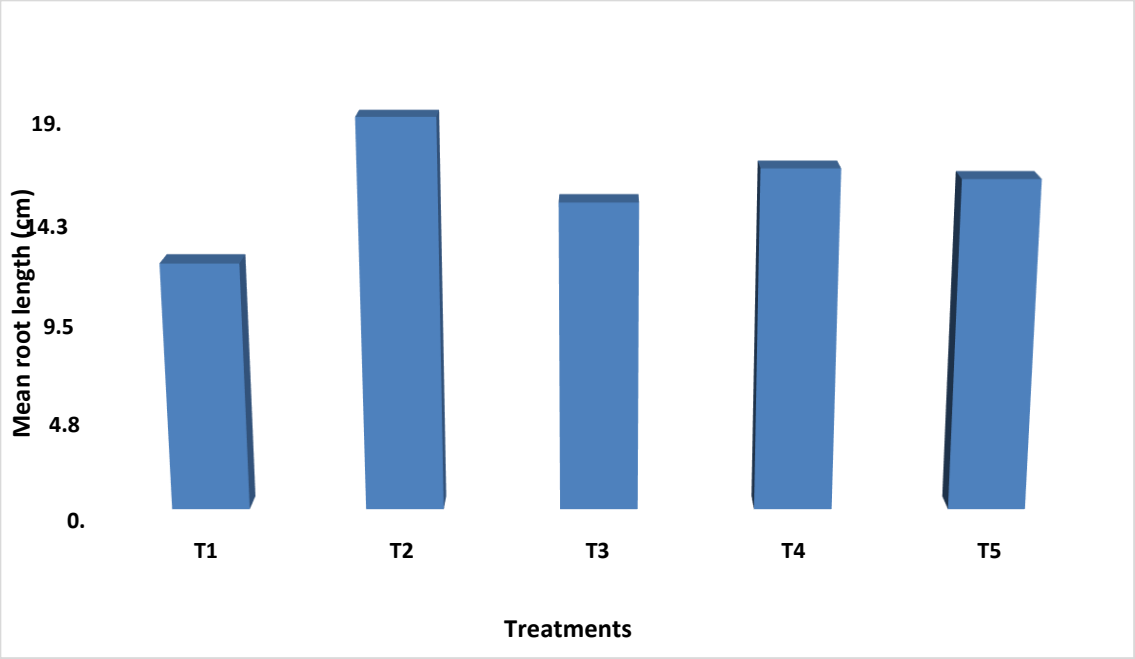

Fig. 5: Mean root length of *K. pinnata* at 8 weeks after planting (WAP)

The root length did not observe a linear increase with increase in concentration of treatment, as was observed in previous parameters studied. Treatment 2 (T2=25% aq. extract) yielded the longest root length. The highest root length was observed for T2 (25% treatment) with a mean root length of 19.0±2.685cm while the lowest was observed for the control (T1) with a mean root length of 12.13±2.71cm. T2 had an approximate 150% increase in root length compared to the control, T1. This shows 1.5 times increase in root length of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Fig. 5).

Effect of treatments on number of roots of *Kalanchoe pinnata*

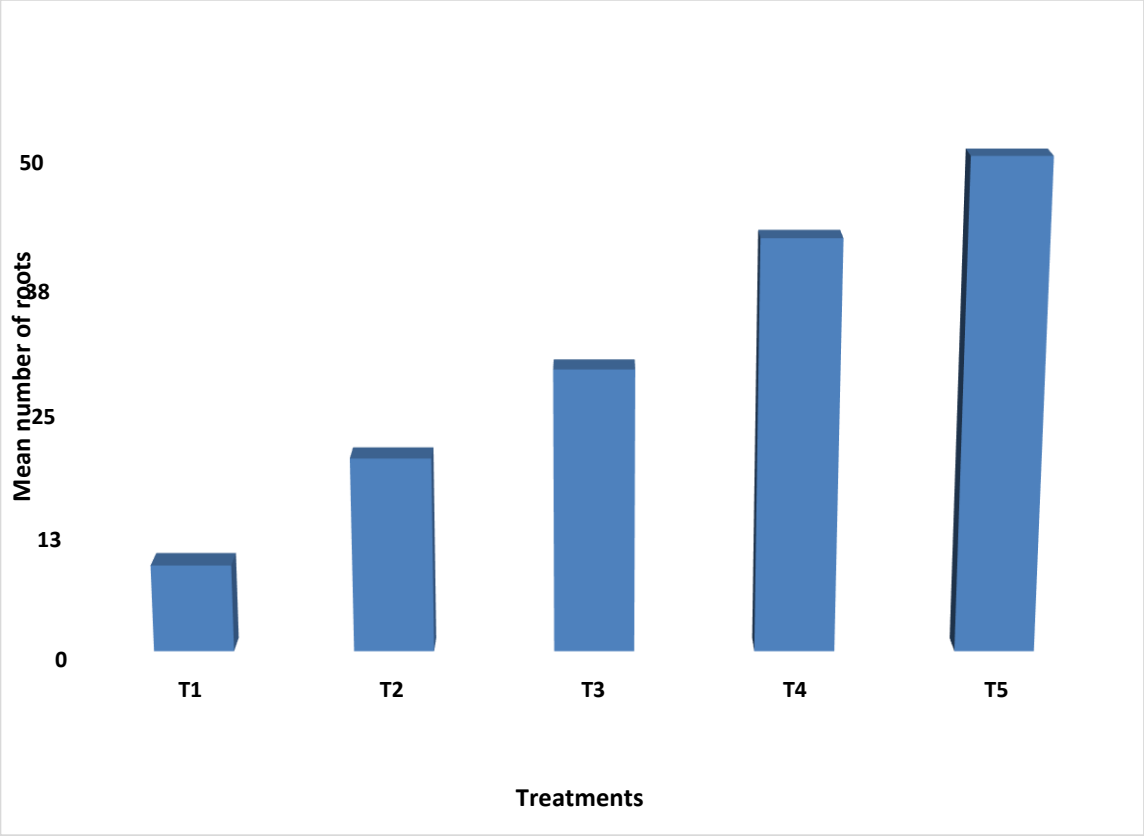

Fig. 6: Mean number of roots of *K. pinnata* at 8 weeks after planting

A linear increase in number of roots of *K. pinnata* was observed with increase in concentration of treatment. The highest number of roots was observed for T5 (100%) with a mean number of roots of 50.0±0.808 while the lowest was observed for the control (T1) with a mean number of roots of 9.03±1.399. T5 (50.0±0.808) had an approximate 500% increase in number of roots compared to the control, T1 (9.03±1.399). This shows 5 times increase in number of roots of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Fig. 6).

Effect of treatments on dry weight of *Kalanchoe pinnata*

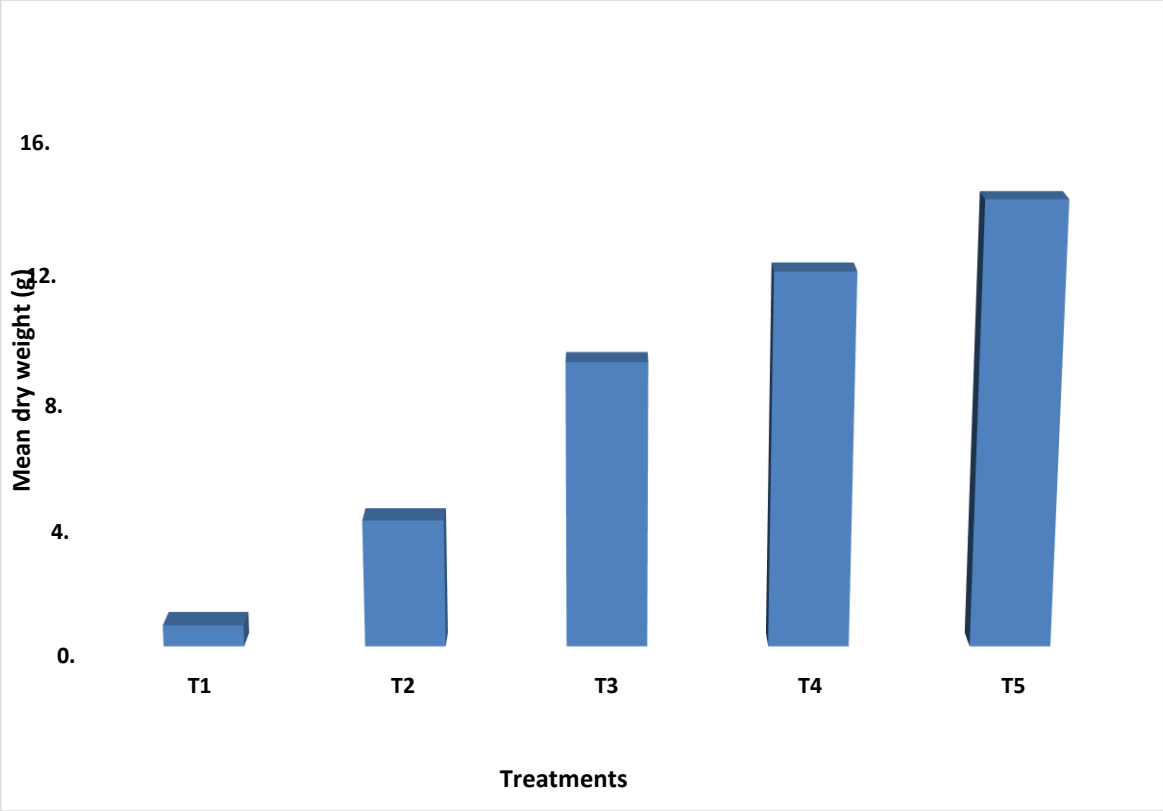

Fig. 7: Mean dry weight of *K. pinnata* at 8 weeks after planting (WAP)

A linear increase in number of roots of *K. pinnata* was observed with increase in concentration of treatment. The dry weight showed a linear increase with increasing treatment. The highest dry weight was observed for T5 (100% treatment) with a mean dry weight of 14.11±0.195g while the lowest was observed for the control (T1) with a mean dry weight of 0.70±0.088g. T5 had an approximate 2000% increase in dry weight compared to the control, T1. This shows 20 times increase in dry weight of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Fig. 7).

Effect of treatments on total chlorophylls content (TCC) of *Kalanchoe pinnata*

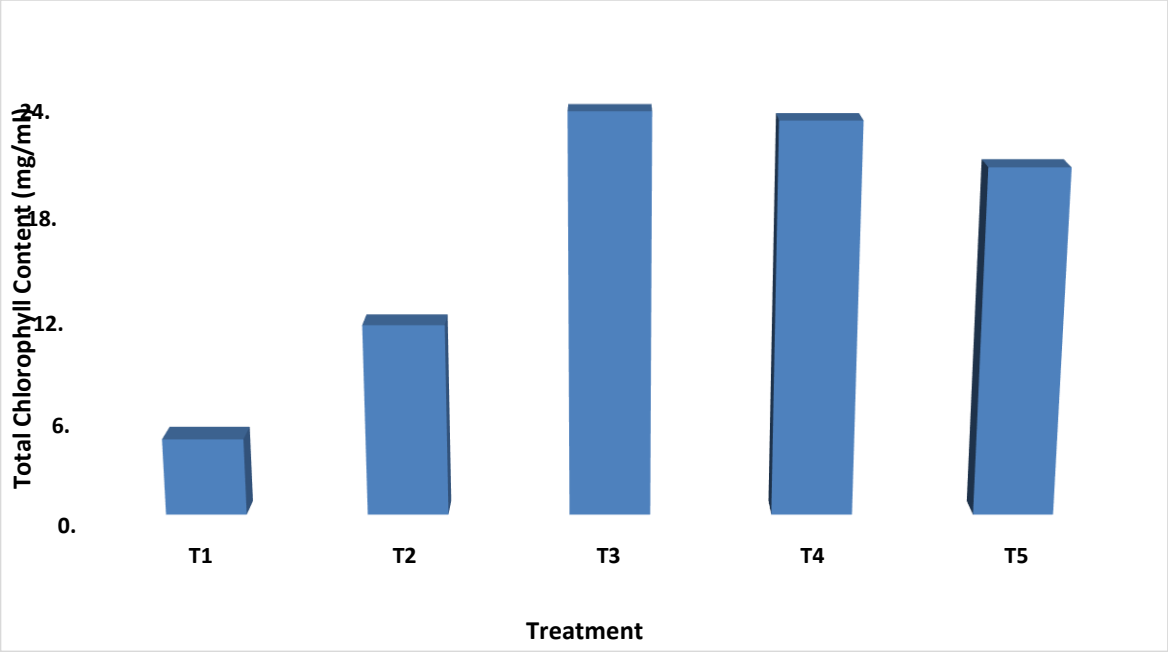

Fig. 8: Mean TCC of *K. pinnata* at 8 weeks after planting

The total chlorophylls content showed a linear increase with increasing treatment up to T3, and then followed a slight downward trend through T4 and T5. The highest total chlorophylls content was observed for T3 (50% treatment) with a mean total chlorophylls content of 23.64±0.3mg/ml while the lowest was observed for the control (T1) with a mean total chlorophylls content of 4.61±0.089mg/ml. T3 had an approximate 500% increase in total chlorophylls content compared to the control, T1. This shows 5 times increase in total chlorophylls content of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Fig. 8).

Effect of treatments on total phenolic content (TPC) of *Kalanchoe pinnata*

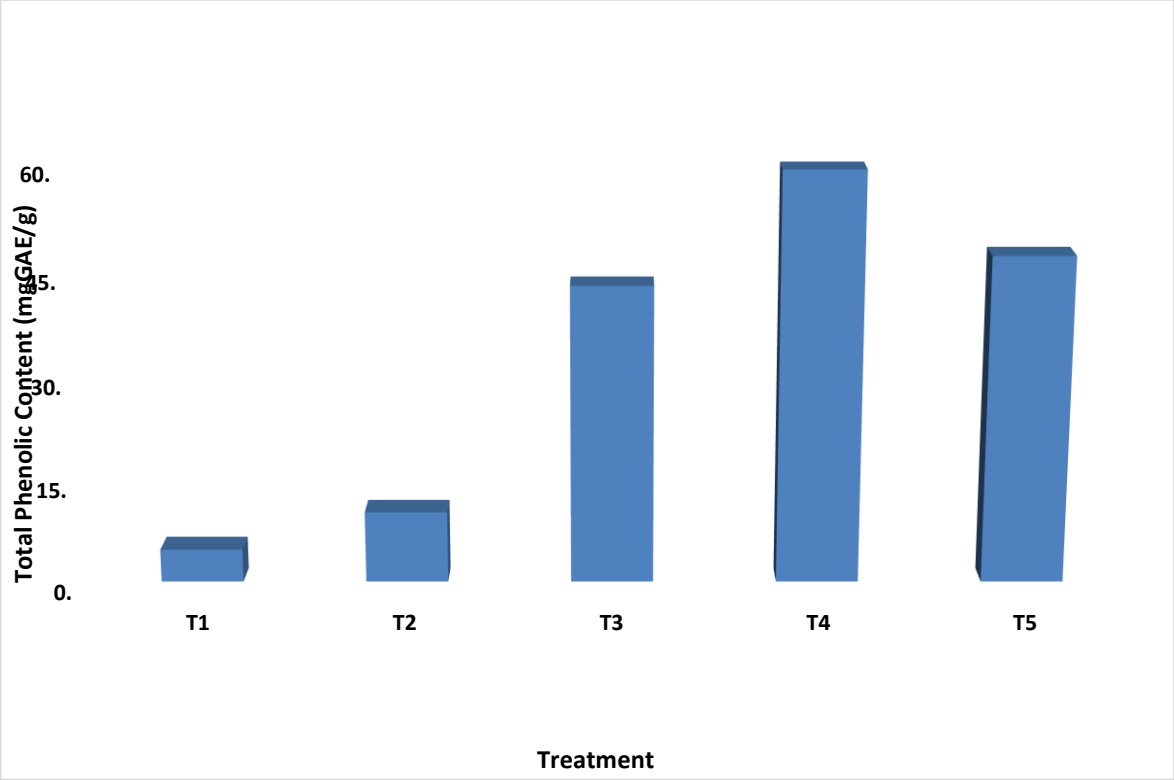

Fig. 9: Mean TPC of *K. pinnata* at 8 weeks after planting

The total phenolic content showed a linear increase with increasing treatment up to T4, and then followed a slight downward trend through T5. The highest total phenolic content was observed for T4 (75% treatment) with a mean total phenolic content of 59.68±0.903mgGAE/g while the lowest was observed for the control (T1) with a mean total phenolic content of 4.76±1.558mgGAE/g. T4 had an approximate 1200% increase in total phenolic content compared to the control, T1. This shows 12 times increase in total phenolic content of *K. pinnata* due to the application of aqueous extract of *Axonopus compressus* in 8 weeks (Fig. 9).

Discussion

All parameters studied (shoot length, number of leaves, leaf area, root length, number of roots, fresh weight, dry weight, chlorophyll-a content, chlorophyll-b content, total chlorophylls content, and total phenolic content) of *Kalanchoe pinnata* were ($P \leq 0.05$) influenced positively by the treatment of aqueous extracts of *Axonopus compressus* as compared to the control. The increase in morphological growth parameters with increase in concentration of treatment may be due to the action of some allelochemicals in the extract that are responsible for cell division. It is also possible that the increase in the growth parameters is due to the stimulatory effect of the allelochemicals on chlorophyllase activity of the plant; and this is in line with the work of Uzoma *et al.,* (2018) who suggested that extracts of *Axonopus compressus* boosted chlorophyllase activity and chlorophylls synthesis in *Kalanchoe pinnata*. This is further supported by the work of Musyimi *et al.,* (2015) who observed that applications of fresh shoot aqueous extracts of *Tithonia diversifolia* increased some morphological growth parameters of *Vigna sinensis*, while suggesting that the stimulatory effect on the morphological growth parameters of *V. sinensis* corresponded to similar increase in total chlorophylls content of *V. sinensis*.

The root length of *K. pinnata* observed its highest value at T2 instead of at T5, quite unlike the trend followed by the other morphological growth parameters studied. This sudden decline in the values of the root length with increase in concentration of treatment goes in line with the reports of Azania *et al*. (2003) who suggested that the plant response to allelochemicals may be concentration-dependent, and could inhibit the growth of some species at certain concentrations while also stimulating the growth of the same or different species at different concentrations. Also, Zhang and Fu (2009) suggested that the inhibition of plant growth parameters at high shoot extracts concentration (100% shoot extracts) may be due to inhibition of cell division and elongation in the plant parts.

The biochemical parameters studied (Total Chlorophylls Content (TCC) and Total Phenolic Content (TPC) also showed a decline at some point with increased concentration of treatment. The biochemical parameters increased linearly with increase in treatment up to a certain point (T3 for TCC and T4 for TPC), and then took a downward trend through T4 and T5 for TCC and T5 for TPC. This

sudden decline in the values of the biochemical parameters with increase in treatment goes in line with the findings of Musyimi *et al*., (2015) who stated that fresh shoot aqueous extracts of *Tithonia diversifolia* have both stimulatory and inhibitory effects on the total chlorophyll content of *Vigna sinensis* which increased significantly with increase in treatment except at 100% treatment where it was inhibited. *Uzoma et* al., (2018) further established the relationship between chlorophyll metabolism and metabolism of phenols using a time lag factor (TLF).

On a general note, aqueous extracts of *A. compressus* was able to significantly ($P \leq 0.05$) increase the morphological growth parameters (shoot length, number of leaves, leaf area, fresh weight, dry weight, number of roots, and root length) and biochemical parameters (total chlorophylls content and total phenolic content) of *K. pinnata* as compared with the control.

Conclusion

All of the parameters studied (morphological growth parameters, chlorophyll content and phenolic content) increased with increase in treatment with aqueous extract of *Axonopus compressus* compared with the control. This showed that *A. compressus* has a positive (stimulatory) allelopathic effect on *Kalanchoe pinnata.* It is therefore advised that *A. compressus* be interplanted with *K. pinnata*, and slashed or mowed *A. compressus* can be used as soil incorporation (manure) in medicinal gardens of *K. pinnata* as this will enhance the growth parameters and boost secondary metabolite production cum medicinal properties of *K. pinnata*, considering its economic uses as an important medicinal plant in Nigeria and Africa at large.

Further work is recommended on establishing the allelopathic effect of *Axonopus compressus* on certain other economic plants, and also on the possible isolation and characterization of the active stimulatory allelochemical from *A. compressus* for its use as a biofertilizer.

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