A Research Article in AJRTC (2014) Vol. 11 No. 1: Pages 10-16

Effects of Aqueous extracts of *Tithonia diversifolia* (Hemsl.) A. Gray, *Vernonia amygdalina* Del. and inorganic fertilizer on the growth and development of cassava (*Manihot esculenta* Crantz).

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Manuscript revised received: 16/05/2014; accepted: 27/06/2014

Abstract
This study investigated the effects of aqueous extracts from fresh leaves of *Tithonia diversifolia* (Hemsl.) A. Gray and *Vernonia amygdalina* Del. as well as inorganic fertilizer on the growth and development of cassava. Six kilograms of fresh and healthy leaves of these plants were washed with tap water, chopped, pounded, soaked for 12hours in distilled water and sieved with 2 mm mesh to obtain their aqueous extracts. 6 week-old cassava plants grown in pots were laid out in a completely randomized design in a Screen House, and were supplied with 500 ml of each extract at 50%w/v and 100%w/v; 1.52 g of NPK fertilizer 15-15-15 while the control was supplied with 500 ml of tap water. The results of the study showed that the aqueous extracts had no significant effects on the growth, development and yield of cassava.

Key words: *Tithonia diversifolia, Vernonia amygdalina,* crude water extract, cassava.

Résumé
Cette étude a été effectuée dans le but de déterminer les effets des extraits bruts dérivés des feuilles fraiches de *Tithonia diversifolia* et *Vernonia amygdalina* et d'un engrais minéral sur la croissance et le développent du manioc. Six kilogrammes de feuilles fraîches et saines de ces plantes ont été lavés à l'eau de robinet, hachés finement, écrasés dans un mortier, imbibés d'eau distillée pendant 12 heures puis pressés et passés à travers un tamis dont les mailles mesurent 2mm afin d'obtenir leurs extraits aqueux. En serre, des plantes de manioc de 6 semaines ont été traitées de façon complètement aléatoire avec 500ml de chacun des deux extraits à 50 et 100% poids/volume, 1,52 g d'engrais inorganique NPK 15-15-15, et 500 ml d'eau de robinet (témoin). Il en ressort que les extraits aqueux bruts de *T. diversifolia* et *V. amygdalina* n'exercent aucun effet significatif sur la croissance, le développement et le rendement en tubercules du manioc en serre.

Introduction
Cassava (*Manihot esculenta* Crantz.) is a common starchy crop grown in most tropical and subtropical regions of the world because of its high energy value. The Food and Agricultural Organization (FAO, 2013) reported that it is the third most important source of calories after rice and maize and it serves as a staple food for about 800 million people in the world. It is cultivated mainly for subsistence living on poor soils often by resource poor farmers (El-Sharkawy, 2004). A remarkable increase of about 60% in the global cassava production has been reported since 2000 thus bringing its production to approximately equal to 280 million tons per annuum (FAO, 2013). In 2011 for example, the most prolific producing countries were Nigeria with 52 million tons (20.8%), Brazil with 25 million tons (10.1 %), Indonesia with 24 million tons (9.5%) and Thailand with 21 million tons (8.7 %)

(FAOSTAT, 2013). In African, Sub-Saharan Africa produces more than half of the global cassava output and this may be due to the numerous advantages this crop has over other cultivated crops in this poverty-stricken part of the world. These advantages include high yields per hectares, tolerance to drought through remarkable physiological adaptations such as rapid stomatal closure, slow soil water depletion (El-Sharkawy 2004, 2006), ability to grow on poor soils, propagation from readily available stem cuttings as well as great flexibility in planting and harvesting. However, in our highly floristically and faunistically diversified tropical/subtropical regions, cassava productivity is susceptible to a number of biotic threats including pests and weeds. Data on the detrimental effects of weeds particularly those belonging to the Asteraceae family on cassava production abound (Eze and Gill 1992; Onwugbuta 2001; Chon *et al*., 2003; Maharjan *et al*., 2007; Ilori *et al*., 2010; Abu-Romman, 2011; Al-Watban and Salama 2012). *Tithonia diversifolia* is an economically important member of this plant family. It is an annual broad-leaved weed which grows to about 5m or more in height. It is common in Nigerian agriculture and has caused farmers to abandon their farmlands due to its prolific growth and it competes aggresively with food crops (Ayeni *et al*., 1997; Tongma *et al*., 1998). On the other hand, it has been reported to stimulate the growth of some crops (Jama *et al*., 2000; Nziguheba *et al*., 2002; Taiwo and Makinde 2005; Oyerinde *et al*., 2009; Ilori *et al*., 2010 and Musyimi *et al*., 2012). *Vernonia amygdalina* (commonly known as bitter leaf), is an important member of this family. It is a bushy shrub or tree of up to seven metres in height. It is widely used as a leafy vegetable in many ethnic groups in the sub Saharan Africa and has numerous ethnoveterinary and ethnomedical uses (Akah and Ekekwe, 1995; Cimanga *et al*., 2004; Igile *et al*., 1995; Argheore *et al*., 1998; Amira and Okubadejo 2007; Yeap *et al*., 2010; Farombi and Owoeye, 2011 and Audu *et al*., 2012).

It is the aim of this study to investigate the possible effects of the aqueous extracts of *T. diversifolia*, *V. amygdalina* and NPK on the growth, development and yield of cassava compared to NPK (15-15-15) alone as a means of utilizing them in soil fertility restoration.

Materials and Methods

Collection of Plant Materials

Fresh and healthy leaves of *T. diversifolia* and *V. amygdalina* were harvested from the Teaching and Research Farm of the Department of Botany, University of Ibadan, Ibadan- Nigeria. The inorganic fertilizer (NPK 15-15-15) and fresh stem cuttings of cassava (TMS 95/0289) used in this study were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan.

Extraction procedure and application of treatments

Six kilograms each of fresh and healthy leaves of *T. diversifolia* and *V. amygdalina* were washed under running tap water, pounded separately using mortar and pestle. The pounded plant materials were separately soaked in 6 litres of tap water for 12 hours and the extracts were obtained by squeezing and sieving through a clean white 2 mm muslin sieve cloth. The extracts obtained served as the aqueous extracts (AEs) of both plants at 100% w/v from which a solution of 50%w/v was prepared by adding an equal volume of tap water. Thus four types of extracts were obtained namely: extracts from leaves of *T. diversifolia* at 100 and 50% w/v (Ti_{100} and Ti_{50}) and extracts from leaves of *V. amygdalina* at 100 and 50 % w/v (Ve_{100} and Ve_{50}).

Experimental setup

Twenty four heavy duty polythene experimental bags were filled with 20 kg of top soil collected from the Teaching and Research Farm of Department of Botany, University of Ibadan, Ibadan. The analysis of the physical and chemical characteristics of the soil was carried out to determine the status of the soil. In each experimental bag, cassava stem cuttings of about 20 cm were half buried in slanting position at an angle of about 45º to the vertical. The plants were left to grow for 6 weeks after which the initial growth parameters (stem girth, leaf length, leaf breadth and plant height) were recorded and the treatments were applied. Leaf length, leaf breadth and plant height were measured with a metre tape and a Mitutoyo Digital Electronic Calliper (MDEC) Model CD.8" was employed in measuring the stem girth. These measurements were taken at alternate weeks (fortnightly). The treatments consisted of 500 ml of each of the extracts (Ti_{100}, Ti_{50}, Ve_{100}, and Ve_{50}) as well as 1.52 g of inorganic fertilizer NPK 15-15-15, while 500 ml of tap water was used for the control. The research design was a completely randomised design with four replicates. The treatments were applied at monthly intervals starting from two weeks after sprouting and establishment (WASE) to harvest. After harvesting, fresh weights of tubers were determined using an OHAUS Advanced Digital Electronic Weighing Balance.

Statistical analysis

Data obtained from the experiment were subjected to one way Analysis of Variance (ANOVA) using SPSS statistical package version 20 and Duncan's Multiple Range Test was used to separate the means ($p \leq 0.05$).

Results

The effects of the aqueous extracts of *T. diversifolia* and *V. amygdalina* on the height of cassava are shown in Table 2. At 6WASE, there was no significant treatment effect on the cassava height except for plants treated with Ve_{50} and NPK. At 10WASE, there were significant effects of aqueous extract of *V. amygdalina* at 50 and 100% respectively (Ve_{50} and Ve_{100}). At 18WASE, there were significant effects of aqueous extract of *T. diversifolia* at 50% (Ti_{50}) and NPK on the cassava height ($p \leq 0.05$). At 20WASE, all treatments had no significant effect on the cassava height.

The effects of aqueous extracts (*T. diversifolia* and *V. amygdalina*) and NPK on stem girth of cassava are as shown in Table 3. Significant effects ($p \leq 0.05$) were observed in plants treated with aqueous extract of *V. amygdalina* at 50% (Ve_{50}) and NPK at 6WASE, while all other treatments were not significant. This trend was repeated over a period of two weeks (8 and 10 WASE). At 12 WASE, only NPK had significant effect on the stem girth ($p \leq 0.05$). However, at 20WASE, Ve_{50} and NPK had significant effects on the stem girth compared to all other treatments ($p \leq 0.05$).

Table 4 showed the effects of aqueous extracts (*T. diversifolia* and *V. amygdalina*) and NPK on leaf length. At 6WASE, there were significant effects of treatments on the cassava leaf length ($p \leq 0.05$). The trend is the same throughout the study except at 16 and 18WASE.

The effects of aqueous extracts (*T. diversifolia* and *V. amygdalina*) and NPK on leaf breadth are shown in Table 5. Significant effects of treatments were observed at 6, 12, and 14WASE ($p \leq 0.05$). At 6WASE, the largest leaf breath was produced by plants treated with NPK while the least leaf breath was produced by plants treated with Ve_{50}. While at 12 and 14WASE, the largest leaf breath were produced by cassava plants treated with NPK. The smallest leaf breath for 12 and 14WASE were produced by cassava plants treated with NPK (18.25±0.48cm) and Ti_{50} (20.33±0.88cm) respectively. It is worthy to note that at 14WASE, no significant effects were observed among the following treatments: Ti_{50} Ve_{50} and NPK. Also, $Ti_{100,}$ Ve_{100} and Control were also not significantly different from each other. However, there were significant treatment effects between $Ti_{50,}$ Ve_{50} and NPK on one hand and Ti_{100}, Ve_{100} and Control on the other hand.

The effects of aqueous extracts *T. diversifolia* and *V. amygdalina* and inorganic fertilizer on cassava yield are presented in Table 6. The highest fresh weight of cassava was obtained in cassava plants from the control (with tap water as treatment) while the plants treated with aqueous extracts of *T. diversifolia* at 50% w/v produced the least weight of tubers. Relatively high yields were obtained in the three following groups: aqueous extracts of *T. diversifolia* at 100% w/v, *V. amygdalina* at 100% w/v and *V. amygdalina* at 50% w/v (Ti_{50}, Ve_{100} and Ve_{50}). Surprisingly, the plants treated with NPK showed relatively low yield.

Table 1: Soil physic-chemical characteristics

Property	Value
Sand (%)	89.4
Silt (%)	3.40
Clay (%)	7.2
pH	6.8
Total Nitrogen (mg/kg)	2.58
Organic Carbon (%)	4.82
Exchangeable Acidity (Cmol/kg)	0.05
Cation Exchange Capacity (Cmol/kg)	6.36
Ca (mg/kg)	2.23
Mg (mg/kg)	2.87
Na (mg/kg)	0.56
K (mg/kg)	0.698
Zinc (mg/kg)	13.45
Copper (mg/kg)	0.59
Iron (mg/kg)	9.4
Manganese (mg/kg)	69.9

Table 2: Effects of aqueous extracts (*T. diversifolia* and *V. Amygdalina*) and NPK (15-15-15) on the plant height (cm)

Treatments	6 WASE	8 WASE	10 WASE	12 WASE	14 WASE	16 WASE	18 WASE	20 WASE
Ti_{50}	40.00±5.90[ab]	69.25±9.20[a]	99.25±13.50[ab]	124.00±16.98[a]	143.00±28.00[a]	156.67±30.48[a]	163.33±33.51[a]	174.33±37.62[a]
Ti_{100}	50.50±4.99[ab]	81.50±5.07[a]	111.00±6.72[ab]	142.00±14.82[a]	166.33±16.97[a]	180.00±17.00[a]	185.33±15.8[ab]	187.00±16.52[a]
Ve_{50}	36.75±4.42[a]	72.75±7.89[a]	84.00±16.67[a]	146.25±15.26[a]	172.25±13.28[a]	171.50±0.87[a]	170.25±4.27[ab]	175.75±5.14[a]
Ve_{100}	56.25±7.19[ab]	93.25±7.70[a]	130.25±8.23[b]	166.25±9.66[a]	180.25±9.96[a]	170.00±13.22[a]	195.50±4.17[ab]	203.75±5.42[a]
NPK	60.75±8.44[b]	89.75±13.03[a]	120.00±15.89[ab]	152.00±19.00[a]	163.25±18.07[a]	197.33±12.41[a]	217.67±5.04[b]	199.00±17.95[a]
Control	46.00±8.82[ab]	81.25±9.95[a]	115.75±10.52[ab]	155.00±14.74[a]	168.25±14.70[a]	181.25±16.55[a]	187.50±17.26[ab]	214.00±20.98[a]

Mean ± SEM (n=4). Values followed by the same superscript within each column are not significantly different according to DMRT ($p \leq 0.05$).

Table 3: Effects of aqueous extracts (*T. diversifolia* and *V. amygdalina*) and NPK on stem girth (mm)

Treatments	6 WASE	8 WASE	10 WASE	12 WASE	14 WASE	16 WASE	18 WASE	20 WASE
Ti_{50}	5.79±0.65[ab]	7.07±0.95[ab]	7.45±0.80[ab]	8.37±0.79[a]	8.90±0.68[a]	9.93±0.97[a]	10.40±0.45[a]	10.39±0.63[ab]
Ti_{100}	5.90±0.63[ab]	6.71±0.64[ab]	8.00±0.55[ab]	8.30±0.76[a]	8.81±0.92[a]	8.74±0.24[a]	10.37±0.49[a]	10.70±0.48[ab]
Ve_{50}	4.85±0.28[a]	5.73±0.17[a]	6.89±0.29[a]	7.72±0.34[a]	8.12±0.47[a]	9.27±0.49[a]	9.87±0.65[a]	9.24±0.72[a]
Ve_{100}	5.89±0.51[ab]	6.80±0.52[ab]	7.77±0.37[ab]	8.93±0.40[a]	9.30±0.30[ab]	10.90±0.53[a]	10.73±0.54[a]	11.43±0.35[ab]
NPK	7.17±0.74[b]	8.08±0.64[b]	9.35±0.46[b]	11.59±0.57[b]	11.27±0.29[b]	11.32±0.78[a]	11.90±0.61[a]	12.11±0.37[b]
Control	5.85±0.94[ab]	6.60±0.80[ab]	7.32±1.00[ab]	8.34±1.27[a]	9.13±1.14[ab]	9.75±1.17[a]	10.42±1.36[a]	11.21±1.14[ab]

Mean ± SEM (n=4). Values followed by the same superscript within each column are not significantly different according to DMRT ($p \leq 0.05$).

Table 4: Effects of aqueous extracts (*T. diversifolia* and *V. amygdalina*) and NPK on leaf length (cm)

Treatments	6 WASE	8 WASE	10 WASE	12 WASE	14 WASE	16 WASE	18 WASE	20 WASE
Ti_{50}	27.00±1.87[ab]	28.25±1.89[a]	35.25±1.80[ab]	36.25±1.75[ab]	41.00±0.58[ab]	44.33±2.03[a]	50.00±6.08[a]	50.33±1.45[ab]
Ti_{100}	29.00±1.47[b]	31.00±1.78[ab]	38.50±0.87[b]	42.50±0.96[c]	45.00±0.58[ab]	48.00±0.58[a]	51.00±1.00[a]	55.00±3.51[b]
Ve_{50}	20.50±1.66[a]	31.00±2.08[ab]	38.75±2.10[b]	41.00±1.73[bc]	41.75±1.80[ab]	45.50±1.71[a]	48.00±1.08[a]	48.75±0.48[a]
Ve_{100}	28.50±1.50[b]	31.00±1.00[ab]	39.25±0.85[b]	41.00±0.71[bc]	47.25±0.48[b]	49.00±0.41[a]	47.00±2.35[a]	49.25±2.29[ab]
NPK	31.75±3.12[b]	33.25±2.87[ab]	29.75±4.25[a]	35.25±1.25[a]	39.75±3.22[a]	47.33±0.88[a]	53.00±3.21[a]	55.33±1.86[b]
Control	26.50±3.30[ab]	37.00±1.00[b]	39.50±1.50[b]	43.50±2.36[c]	47.25±2.56[b]	49.25±2.56[a]	50.75±2.06[a]	52.75±0.85[ab]

Mean ± SEM (n=4). Values followed by the same superscript within each column are not significantly different according to DMRT ($p \leq 0.05$).

Table 5: Effects of aqueous extracts (*T. diversifolia* and *V. amygdalina*) and NPK on leaf breadth (cm)

Treatments	6 WASE	8 WASE	10 WASE	12 WASE	14 WASE	16 WASE	18 WASE	20 WASE
Ti_{50}	14.75±0.48[ab]	15.50±1.32[a]	18.75±0.75[a]	19.75±0.85[ab]	20.33±0.88[a]	24.67±1.45[a]	28.33±3.18[a]	27.67±1.20[a]
Ti_{100}	15.50±0.29[ab]	17.25±0.63[a]	17.75±0.85[a]	21.25±0.75[abc]	25.00±1.15[b]	27.67±0.88[a]	29.67±0.67[a]	30.67±0.67[a]
Ve_{50}	12.50±0.87[a]	18.00±1.08[a]	18.50±0.87[a]	20.75±0.85[abc]	20.75±1.11[a]	24.75±1.38[a]	26.25±1.49[a]	26.00±3.76[a]
Ve_{100}	15.75±1.03[ab]	17.75±1.31[a]	20.25±0.63[a]	21.75±0.63[bc]	25.25±0.48[b]	26.00±1.08[a]	27.75±1.49[a]	27.75±1.18[a]
NPK	16.75±1.49[b]	18.75±1.25[a]	17.50±0.29[a]	18.25±0.48[a]	21.00±1.29[a]	24.67±0.88[a]	29.67±1.45[a]	32.67±0.88[a]
Control	15.75±1.93[ab]	18.50±1.50[a]	20.00±2.00[a]	23.75±1.75[c]	26.00±1.22[b]	28.00±0.91[a]	27.50±1.26[a]	29.50±1.50[a]

Mean ±SEM (n=4). Values followed by the same superscript within each column are not significantly different according to DMRT ($p \leq 0.05$).

Table 6: Effects of aqueous extracts *T. diversifolia* and *V. amygdalina* and Inorganic fertilizer on cassava yield

Treatments	Mean ± SEM
Ti 50	15.23±8.53[a]
Ti 100	29.45±3.65[ab]
Ve 50	28.92±3.22[ab]
Ve 100	26.15±7.55[ab]
NPK	11.43±2.61[a]
Control	40.30±12.80[b]

Mean ± SEM (n=4). Values within each column with the same superscript are not significantly different ($p \leq 0.05$) using DMRT

Discussion

There was a gradual increase in the height of the cassava plants with time and age. This was evident in Table 2. However, the different aqueous extracts of *T. diversifolia* and *V. amygdalina* had no significant treatment effect on the height of cassava. This result did not agree with the results of Oyerinde *et al*., (2009) who reported that the fresh aqueous shoot extracts of *T. diversifolia* stimulate the growth of maize at an advanced growth stage. Taiwo and Makinde (2005) similarly reported that the leaves of *T. diversifolia* enhance the growth of cowpea when incorporated into the soil. However this study agreed with Ilori *et al*. (2007) who reported that aqueous extracts obtained from different parts of *T. diversifolia* are phytotoxic and retarded the shoot growth of rice. They further stated that inhibition was more pronounced in older rice plants. The present study adds to the growing number of contradicting literature with respect to the potentials of *T. diversifolia* as plant growth stimulator. However, it is important to note that these differing results may be due to the differences in the test plants and climate in which these studies were conducted and the plant parts from which the extracts were prepared, the solution used in the extraction and the concentration of the extracts as well as several other factors such as plant growth conditions.

It is interesting to note that there is dearth of research reports on plants stem girth as a growth parameter. This study offers a reliable and easy-to-quantify growth parameter as an index for measuring plant growth performance. Table 3 showed that the cassava stem girth was not significantly different under the different aqueous extracts regimes. However mean comparison revealed that the inorganic fertilizer impacted positively on the stem girth of the test plant.

Leaves are one of the most important organs of green plants (Canton and Venus 1981). The rate of photosynthesis depends on the amount of light intercepted by a leaf which in turn is closely related to the dimensions of the leaf. The leaf length and leaf breadth of the cassava plants studied responded positively to the aqueous extract treatments given to them (Tables 4 and 5). Also, the inorganic fertilizer showed stimulating effects on both growth parameters.

The fresh weight of tuber (yield) showed that the treatment effects were significantly different from the control. This showed that the aqueous extracts *T. diversifolia* and *V. amygdalina* and inorganic fertilizer inhibited cassava yield. This study did not agree with the works of Jama *et al*., (2000) and Nziguheba *et al*., (2002) that used *T. diversifolia* compost as a rich nutrient source in soil fertility restoration and reported higher yield compared to conventional fertilizers.

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