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Isolation and Identification of Some Plant Growth Promoting Substances in Compost and Co-Compost

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Abstract: In this study, plant growth hormones in compost or co-compost prepared from human excreta and/or municipal solid waste were extracted and identified. Water (deionized) or 80% methanol (chilled) extracts were prepared from the composts for the isolation and identification of plant growth substances. The methanolic extracts of plant growth hormones fraction was further partitioned into cytokinin (fraction) using ethyl acetate and n-butanol; auxins (fraction) using diethyl ether and gibberellins (fraction) using sodium hydrogen carbonate and n-butanol. Five different concentrations of standard plant growth hormones; Benzyl Amino Purine (BAP), Gibberellic Acid (GA_3) and Indole-3-Acetic Acid (IAA) were prepared and used in a bioassay test to compare the effects and concentration of the extracted plant growth hormones from the compost and co-compost. The presence of some plant growth hormones in the extracted samples from compost or co-compost was confirmed using the relative fluidity, (Rf) values of the co-chromatography using a standard hormone and the extracted plant growth hormones. The results indicated the presence of plant growth substances such as IAA, GA3 and cytokinins. In co-compost, the concentration of hormone were found to be 68.3 to 345.1 mg kg^{-1}, 61.9 to 185.8 mg kg^{-1} and 250.4 to 312.7 mg kg^{-1} for auxins, cytokinins and gibberellins, respectively. Whereas in compost 42.0 to 248.8 mg kg^{-1}, 33.1 to 198.3 mg kg^{-1} and 10.1 to 200.2 mg kg^{-1} of auxins, cytokinins and gibberellins, respectively were found.

Key words: Compost, co-compost, bioassay, municipal solid waste, plant growth hormones

INTRODUCTION

Waste management is a problem to municipal authorities in many developing countries where the facilities available to municipal authorities for collection of waste are usually not enough as well as inadequate. As a result, wastes are seen choking storm drainages, on the streets, scattered around collection points or are dumped at unauthorized places which eventually pose a threat to the environment and human health. There is therefore the need to develop alternative approaches or methods of managing waste in developing countries. One of such approaches of waste management is to convert waste into compost/co-compost which is reported to support plant growth (Nardi *et al*., 1994). Compost prepared from municipal solid waste and co-compost, municipal solid waste blended with human excreta are made up of humic substances that contribute to plant growth and development (Piccolo *et al*., 1992; Chen and Aviad, 1990). Generally, the application of organic waste to the soil causes an increase in soil chemical, physical and biological properties (Tejada and Gonzalez, 2004).

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The significant growth exhibited by plants grown in compost/co-compost suggests that compost/co-compost provide more than just plant nutrients (nitrogen, phosphorus and potassium) for growth (Atiyeh *et al*., 2000).

It is envisaged that compost/co-compost probably contains plant growth hormones, although this has not been firmly established. Whereas other authors believe that the plant tissues cannot store these plant growth hormones due to their unstable nature, another school of thought also proposes that plant growth hormones are also present in compost, but are synthesized by microorganism in the compost (Pizzeghello *et al*., 2001; Glick, 2003). Another group of workers also proposed that the humic acid and fulvic acid present in the compost/co-compost rather contain or behave like plant growth hormones (Nardi *et al*., 1994). However, it appears that there has not been much attention given to the extraction and identification of the hormones from compost/co-compost.

Consequently, this study was carried out to isolate and identify plant growth hormones present in compost and co-compost.

MATERIALS AND METHODS

Extraction of Plant Growth Hormones from Recycled Waste Materials

The study was carried out at Ecological Laboratory of the University of Ghana, Legon between August, 2006-May, 2007 to isolate and identify plant growth hormones in co-compost and compost. Plant growth hormones were extracted from samples of municipal solid waste materials which have been recycled into compost and dewatered human excreta mixed with the recycled municipal solid waste to form co-compost. Samples of the compost and co-compost were obtained from a composting site at Buobai near Kumasi. Distilled water was first used to extract the plant growth hormones from the compost and the co-compost, followed by the use of chilled 80% methanol.

Water Extraction

The procedure described by Witham *et al*. (1971) was used in the extraction. Ten grams of the material (either compost or co-compost alone) was put into extraction bottle and 150 mL of distilled water was added. The slurry was placed on a mechanical shaker for approximately 3 h. The water extract was allowed to stand and partitioned into solid and liquid phases, after which the supernatant was decanted and centrifuged for 6 min at 4000 rpm. The same volume of distilled water was added to the solid residue in the extraction bottle shaken for 3 h on the mechanical shaker before decanting and centrifuged at the same time and speed. This process/procedure was repeated a third time and the total volume of the extracts was bulked. In all 60 g of the ground materials was extracted with 900 mL of distilled water.

Eighty Percent (80%) Methanol Extraction

In order to obtain a high concentration of any potential plant growth hormones present in compost or co-compost, the procedure described by Badr *et al*. (1971) and modified by Taylor *et al*. (2004) was adopted. Fifteen grams of either compost or co-compost alone was weighed into an extraction bottle and 150 mL of chilled 80% methanol was added. This process was repeated six times to obtain a final/ total volume of 900 mL of 80% methanol. One hundred and fifty mililitres of 80% methanolic slurry of either compost or co-compost alone was placed on a mechanical shaker for approximately 24 h. The slurry was allowed to stand and partitioned/separated out into the liquid and solid phases prior to decanting the supernatant. The filtrate was then centrifuged for 6 min at 4000 rpm, filtered and stored at -5°C in a freezer. The same volume of 80% methanol was added to the residue in the extraction bottle and shaken for another 24 h after which the solution was again

decanted, centrifuged for 6 min at 4000 rpm and filtered. The extraction process was repeated a third time following which all the extracts were bulked and reduced to aqueous phase (48 mL) using a rotary evaporator prior to storage in a refrigerator.

Solvent Partitioning

The pH of the aqueous extract from either the compost or co-compost alone was adjusted to 2.5 by adding few drops of 1N H_2SO_4. The acidified aqueous extract (48 mL) was transferred into a separatory funnel and 380 mL of ethyl acetate was added, shaken and allowed to partition into the organic and aqueous phases. The organic phase (ethyl acetate) was separated from the aqueous phase into a clean conical flask before the same volume of ethyl acetate (45 mL) was again added to the aqueous phase, shaken, allowed to stand and the organic phase separated from the aqueous phase. This process was repeated a third time, before the three (3) aliquot of the organic phases were pooled together. The pH of the remaining aqueous phase was adjusted to 7 by adding a few drops of 1 N NaOH. This was then transferred into separatory funnel and the same volume of water saturated n-butanol (40 mL) added. The funnel was shaken gently and the solution allowed to separate into less dense water saturated n-butanol phase (organic) and denser aqueous phases, before the two phases were separated. This process was repeated three times using the aqueous phase. All the water saturated n-butanol organic phases (which were presumed to contain the cytokinins) were bulked, stored at -5°C for further analysis and the aqueous phase was discarded. The ethyl acetate phase (125 mL) was also transferred into a separatory funnel and an equal volume of 5% $NaHCO_3$ was added, shaken and allowed to partition into organic (ethyl acetate) and aqueous ($NaHCO_3$) phases. The two phases were separated from each other and the process was repeated four times. All the aqueous phases were bulked, stored at -5°C and the organic phases (ethyl acetate) were discarded. The aqueous phase was adjusted to pH 2.5 with drops of 1 N H_2SO_4. It was transferred into a separatory funnel and an equal volume (45 mL) of dry diethyl ether was added. The separatory funnel was shaken and the solution separated into organic (diethyl ether) and aqueous ($NaHCO_3$) phases on standing before the aqueous phase was separated from the organic phase. This step was repeated four times with the aqueous phase and each phase bulked separately and stored at -5°C. The ether phase was presumed to contain auxins. The stored acidic aqueous phase was poured into a separatory funnel and the same volume (45 mL) of water saturated n-butanol was added. The content was shaken and allowed to partition into organic (n-butanol phase) and aqueous phases before the two phases were separated. The process was repeated four times using the aqueous phase. All the organic phase or the water saturated n-butanol phase were combined and stored at -5°C in a refrigerator (this was presumed to contain gibberellins). The aqueous phase obtained was discarded. All the partitioned stored extracts were dried at 40°C using a rotary evaporator. These samples were then subjected to thin layer chromatography for further purification and identification, bioassay to determine the concentration of each extracted plant growth hormones and spectra analysis.

Identification of the Plant Growth Hormones

Identification of plant growth hormones was done using bioassay, co-chromatography (Co-TLC), colour of sprayed spots with reagents and spectrophotometer spectral bands.

Bioassay Using Water Extract

Serial dilutions of the water extract (100%) were prepared to obtain C75 (75 mL extract +25 mL distilled water), C50 (50 mL extract +50 mL distilled water), C25 (25 mL extract +75 mL distilled water) solutions. Distilled water was used as control. Three petri dishes were prepared for each concentration and the control. Each petri dish was lined with filter paper and 10 grains of maize variety (Obaatanpa) were placed in the dish and replicated three times. Five millilitres of the diluted extract and the control (distilled water) was added to the corresponding labelled petri dish. Elongation of the radicle and coleoptiles was measured at 24 h intervals.

Co-Chromatography of Extracts and their Standards

Dried extracts from the solvent partitioning were dissolved in 1 mL of methanol, except the cytokinins fraction which was dissolved in 80% methanol Standards for plant growth hormones: Benzyl Amino Purine (BAP), Indole-3-Acetic Acid (IAA) and Gibberelleric Acid (GA_3) for each extract were also prepared. Each extract and its prepared standard were spot loaded onto thin layer chromatographic plates of dimension 20×20 cm and silica gel (60_{254}) of thickness 0.25 mm and developed in isopropanol: ammonium hydroxide: water (84:4:4 v/v/v) to about eighteen centimeters (18 cm) in a vertical direction, except for the cytokinins which were run in butanol:ethyl acetate: water (90:10:10 v/v/v) solvent system. The distance moved by the solvent system (solvent front) and the spots were measured, after which the relative fluidity, R_f values were calculated by dividing the distance moved by the solvent system by the distance moved by the spots. The R_f values of the extracted plant growth hormones were compared to the R_f values of the standard hormones.

Colour Reactions of the Separated Plant Growth Hormones from the Co-Chromatography

Following the procedure described by Herborne (1998) for identification of cytokinins, each developed TLC plate containing the separated spots of the standard and extracted hormones was sprayed with bromophenol blue, observed when dried and again observed under the UV light or wavelength 254 nm. Furthermore, another developed TLC plates containing the separated spots from both the standard and extracted hormones were each sprayed with H_2SO_4: water (7:3) and heated at 120°C. The plates were then observed under the UV light at wavelength 254 nm (Unyayar *et al*., 2002). The other spot separated spots from the TLC were exposed to ammonia vapour to form ammonia complex. The plates were then observed under the UV light at a wavelength of 254 nm.

Determination of Concentrations of Plant Growth Hormones in Co-Compost/Compost Using Bioassay

The methods used by Hedden (1993) and Witham *et al*. (1971) were employed to determine the concentration of plant growth hormones in the compost and co-compost.

The dried extracts were each dissolved in 2 mL of methanol and diluted to different solutions of unknown concentrations with deionized water (test solution). Standard GA_3, BAP and IAA of different concentrations; 100 ppm (10^{-4} M), 10 ppm (10^{-5} M), 1 ppm (10^{-6} M), 0.1 ppm (10^{-7} M), 0.01 ppm (10^{-8}M) were prepared. Methanol and deionzed water were used as controls. Petri dishes were lined with filter paper and arranged three each for a treatment in three replicates. There were a total of 72 petri dishes for the test solution, 45 dishes for the standard hormones and 18 for the controls.

Maize grains were soaked in tap water for about five hours to remove any dirt or greasy substances. Fifteen grains of maize were placed in each petri dish and placed in the dark at 25°C and 85-95% humidity to germinate. Two days after germination, 10 uniform seedlings were selected from each petri dish and 3 mL of each treatment solution was added to each petri dish. The length of coleoptile, radicle and weight of cotyledon (cytokinin) were measured after 5 days of growth in continuous florescence light. The concentrations of the standard IAA and GA_3 were plotted against coleoptile length, while the concentrations of the BAP were plotted against the weight of cotyledon to obtain standard response curves. The concentration of each test solution was determined from the standard curves.

RESULTS

Bioassay for Identification of Plant Growth Hormones

Figure 1-4 show the effect of water extract of compost and co-compost diluted to different concentrations, on the elongation of radicle and coleoptile of maize. There were significant differences among treatments in the length of coleoptile and radicle of the seedlings treated with C100, C75, C50,

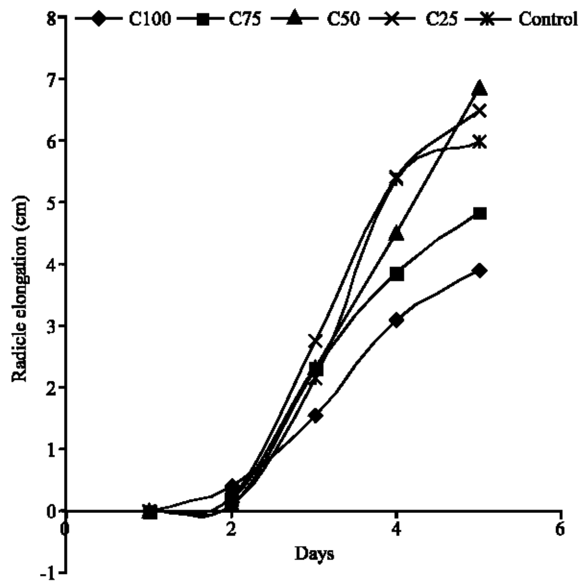

Fig. 1: Radicle elongation of maize treated with water extract from compost

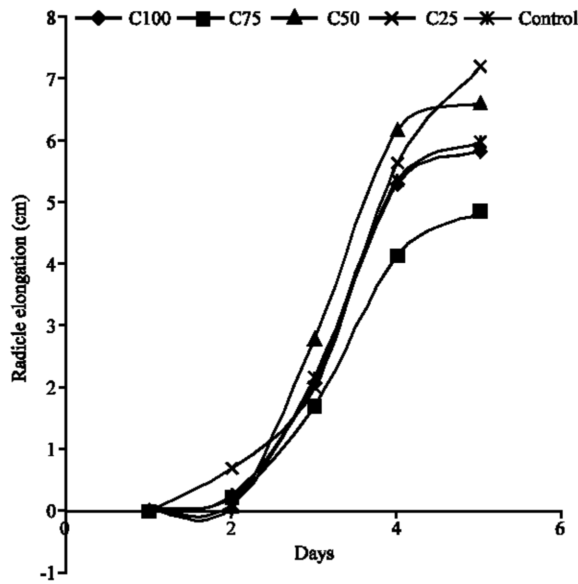

Fig. 2: Radicle elongation of maize treated with water extract from co-compost

C25 and the control ($p<0.05$). It was observed that C25 the most diluted solution of the extracts stimulated coleoptile and radicle elongation more than the other solutions (Fig. 2-4) except in Fig. 1 where C50 performed better than C25. The highly concentrated solutions, C75 and C100, inhibited coleoptile and radicle elongation.

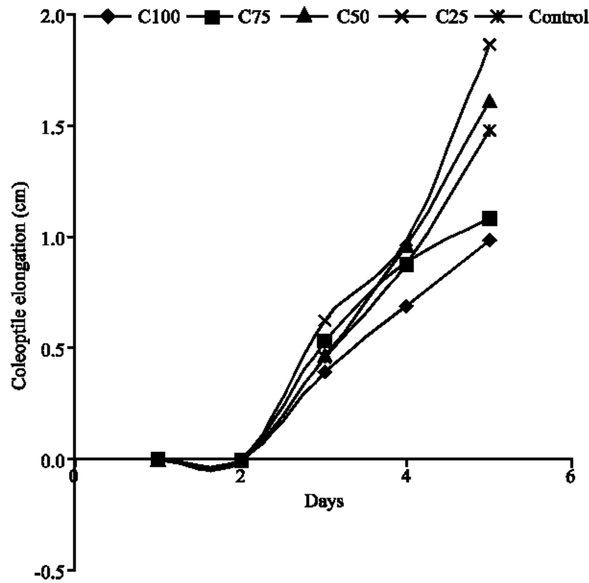

Fig. 3: Coleoptile elongation of maize treated with water extracts from compost

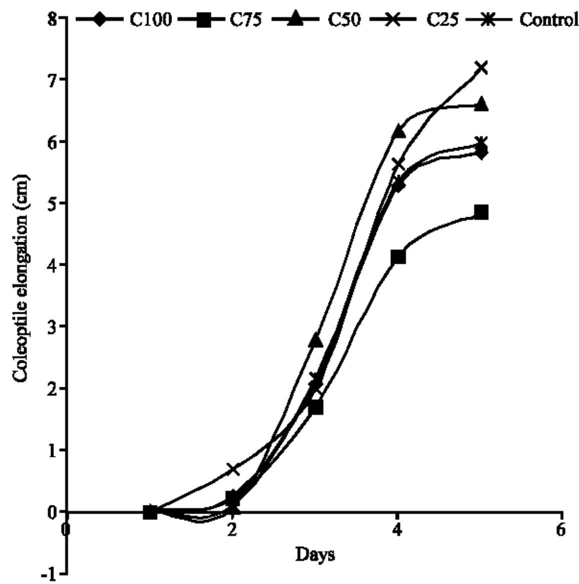

Fig. 4: Coleoptile elongation of maize treated with water extracts from co-compost

Confirmatory Test for the Separated Plant Growth Hormones

Table 1 shows the calculated Rf values, colour reactions of isolated spots treated with the vapour of ammonia, bromophenol blue and H_2SO_4: water (7:3 v/v).

The co-compost extract of auxin gave two R_f zones on TLC whiles the compost extract gave only one. One of the R_f zones from the co-compost extract (spot 2) had R_f value, 0.8475 which was close to the R_f value of the standard IAA (0.8203). This spot also produced the same colours as the standard when treated with Bromophenol blue and ammonia. The other R_f zones also produced colours similar to that of the standard. The co-compost and compost extracts of cytokinins produced three and two

Table 1: Co-chromatographic and colour formation from reagents result (Confirmatory test)

Hormones	Solvent front (cm)	Spot distance (cm)	No. of spots	R_f values	Colour of spots with Bromophenol blue spray	Colour of spots with ammonia spray	Colour of spots with H_2SO_4/H_2O (9/1 v/v) spray
Auxins from co-compost	16.00	14.2	2	0.8475	Light blue	Brown	Light brown
		10.8		0.6750	Pale blue	Brown	Fluorescence light green
Auxins from compost	16.00	12.4	1	0.7750	Light blue	Light yellow brown	Light brown
Standard IAA	16.00	13.1	1	0.8203	Pale blue	Brown	Bluish brown
Cytokinins from co-compost	16.40	11.8	3	0.7235	Greenish blue	Deep blue	Fluorescence green
		10.3		0.6340	Fluorescence blue	Deep blue	Light yellow
		9.2		0.5576	Light blue	Blue	Reddish yellow
Cytokinins from compost	16.45	11.7	2	0.7156	Deep blue	Bluish brown	Light yellow
		5.4		0.3285	Light blue	Light yellow	Fluorescence light green
Standard BAP	16.40	12.7	1	0.7765	Deep blue	Light yellow	Greenish brown
Gibberellins from co-compost	16.30	14.8	3	0.9067	Greenish blue	Bluish brown	Yellow-green
		8.6		0.5267	Brownish blue	Brown	Orange green
		6.7		0.4133	Fluorescence blue	Bluish brown	Pale yellow
Gibberellins from compost	16.30	7.1	2	0.4334	Light blue	Yellowish brown	Light yellow
		3.5		0.2134	Greenish blue		Yellow-brown
Standard GA_3	16.30	12.1	1	0.7376	Greenish blue	Brown	Yellow-green

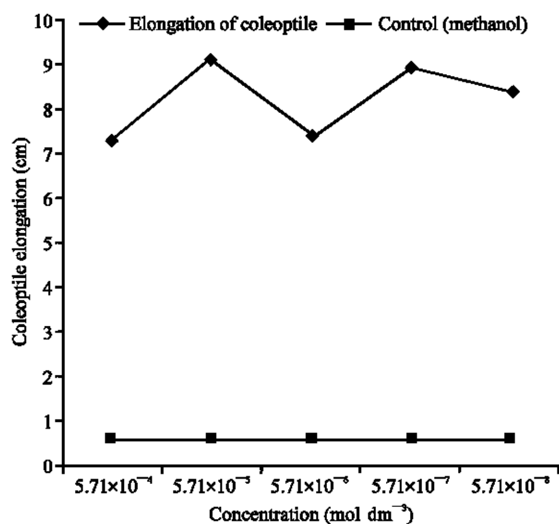

Fig. 5: Standard curve showing the effect of IAA on elongation of coleoptile of maize

spots, respectively. One spot each from both the co-compost (spot 3) and the compost (spot 2) produced R_f values of 0.7235 and 0.7156, respectively, as compared to the R_f value of 0.7765 from the standard, BAP. All these spots produced blue colouration with bromophenol blue. In another development, the spots from the gibberellins gave R_f values that were different from the standard, GA_3. However spot 1 from co-compost (R_f value 0.4133) and spot 2 from compost (R_f value 0.4334) were almost the same and produced a yellow colour as compared to the yellow to green colour of the standard.

Determination of Concentrations of Plant Growth Hormones in Co-Compost and Compost Using Bioassay

Figure 5 shows the responses of coleoptile elongation to the various concentrations of auxins (standard). The solution with concentration of 5.71×10^{-5} M stimulated coleoptile elongation more than

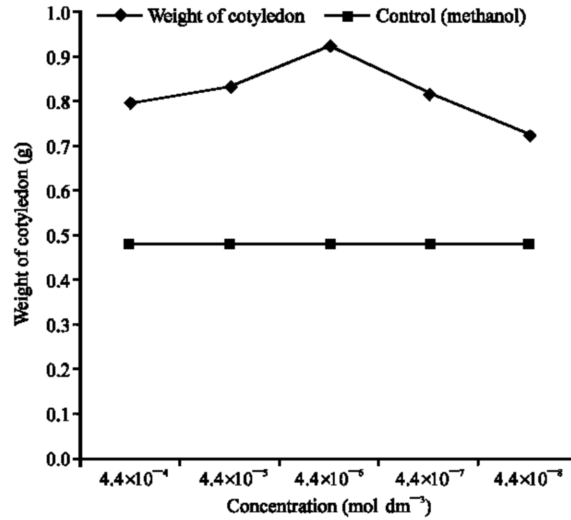

Fig. 6: Standard curve showing the effect of BAP on weight of maize cotyledon

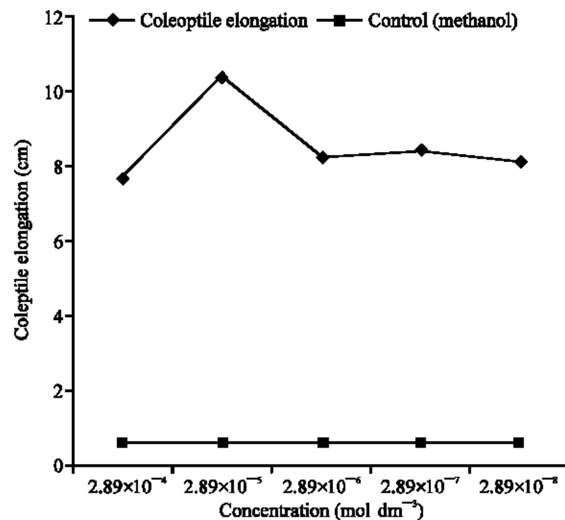

Fig. 7: Standard curve showing the effect of GA3 on elongation of coleoptile of maize

the other solutions. The standard curve was used to calculate the auxin concentration in the extracts of the compost and co-compost.

The auxins concentration was estimated from the curve to be 68.3 to 345.1 mg kg^{-1} in the co-compost and 42.0 to 248.8 mg kg^{-1} in the compost.

Figure 6 also shows the response of weight of cotyledon to various concentrations of BAP (standard). The 1.47×10^{-6} M solution responded to highest production of weight of cotyledon than the remaining solutions. Again the standard curve was used to calculate the cytokinin concentration in the extracts of the compost and co-compost.

The concentration of cytokinins in co-compost was found to be 61.9 to 185.8 mg kg^{-1} and 33.1 to 198.3 mg kg^{-1} in the compost.

Figure 7 shows the response of coleoptile elongation to the various concentrations of gibberellins (standard). The solution with concentration of 5.71×10^{-5} M stimulated coleoptile elongation more than

the other solutions and a standard curve was used to calculate the gibberellins concentration in the extracts of the compost as well as the co-compost. The extracted gibberellins from co-compost and compost were estimated as, 250.4 to 312.7 and 10.1 to 200.2 mg kg^{-1}, respectively.

DISCUSSION

Methanol was used in the extraction of the plant hormones since alcohol is a good all purpose solvent for preliminary extraction because it can extract both polar and non-polar constituents (Harborne, 1998). Piccolo *et al*. (1992) reported that the constituents of compost or co-compost which are humins, fulvic acid and humic acid, each dissolves in different solvents at different pH. Humins are not soluble in alkali (high pH), acid (low pH) and in water (at any pH) since they are considered macro organic substances due to their higher molecular weight. Humic acids are organic acids which are soluble in water under alkaline condition only and acid is precipitated in aqueous solution under acidic condition. Fulvic acid on the other hand, is soluble in water under all pHs since it has more hydrophilic (water-loving) end than lypophilic. Thus, 80% methanol stands to be the best solvent since it is a polar organic solvent which can dissolve both organic and inorganic substances. Partitioning the extracts into acidic, neutral and basic pHs enabled the dissolution of the basic, neutral and acidic components in the compost or co-compost such as humins, humic acid, fulvic acid and their hormonal content (Harborne, 1998).

Of the several solvent systems used for the thin layer chromatography, Isopropanol: Ammonium hydroxide: Water (90/10/10, v/v/v) gave the best separation for auxins and gibberellins (Taylor *et al*., 2004). Butanol: acetic acid: water (80/10/10, v/v/v) was selected for cytokinins since it proved to be the best solvent system. Thin layer chromatography apart from being used for purification and identification of compounds also gives an idea of the number of components present in a sample (Sherma, 2002).

For auxins, two spots and one spot were identified from the co-compost and compost, respectively. Gibberellins and cytokinins both gave three well separated spots each for co-compost as compared to two each in the compost extracts.

The R_f (relative fluidity or Retardation factor) can be used to identify the types of component or plant hormones present in an extract (Sherma, 2002). Identification of the plant hormones was based on co-chromatography with the authentic plant hormones. IAA standard produced R_f value of 0.8203 as compared to 0.8475 of the extracted auxin spot from R_f zone 2 of the co-compost. This indicates that the co-compost contains a hormone which is similar to the IAA standard. Similarly, the R_f values of the separated components from co-compost (0.7235) and compost (0.7156) of the extract of cytokinins, compared favourably to the standard BAP (0.7765). The R_f values for co-compost and compost separated spots were not similar to the R_f value (0.7376) of the standard gibberllins (GA_3) and implies that the isolated hormones may be other forms of gibberellins other than GA_3.

The types of hormones extracted were confirmed by the colour of the spot of hormones sprayed with bromophenol blue, sulphuric acid: water (7:3, v/v) and exposure to ammonia and observed under UV light. The auxins spots from co-compost and compost gave different shades of brown colour with ammonia complex which closely matched the deep brown colour from the standard (IAA). The standard, GA_3 and BAP produced colours which matched TLC spots from compost and co-compost. According to Harborne (1998), cytokinins can be detected as blue spots after spraying with bromophenol blue or silver nitrate reagent. Similarly, the standard method of detecting gibberellins is by spraying plates of the separated spots or chromatogram with sulphuric acid-water (7:3, v/v) and heating at 120°C; gibberellins appear as yellow-green spots (Harborne, 1998).

The most diluted water extracts from the compost and co-compost were more effective at stimulating growth than the other diluted extracts. The 25% concentration of the water extract was the

most effective in stimulating the elongation of coleoptiles and radicles in both maize and cowpea. Plant growth hormones are required in minute quantities to elicit particular response (Moore *et al*., 1995). The extracts from both compost and co-compost diluted to 25% concentration might have contained the optimal amount of plant growth hormones needed to stimulate the elongation of coleoptile and radicle in both maize and cowpea.

CONCLUSION

The study confirmed the presence of auxins, gibberellins and cytokinins in both co-compost and compost. The effect of compost and co-compost on crop growth and development may be the result of the interaction between the nutrients present and plant growth hormones.

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