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RESEARCH ARTICLE

SYNTHESIS, PURIFICATION AND CHARACTERIZATION OF C-CONTAINING NANOPARTICLES AND ORGANIC COMPOUNDS RICH IN ORGANIC COMPOSTING

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ABSTRACT

A study was conducted by decomposing of *Parthenium hysterophorus* (Linn.) weed plants with farm yard waste; maize waste and chick waste in the ratio3:4:2:1 which allowed on an anaerobic fermentation fraction of the C-containing organic colloids of nanoparticles and organic compounds. The carbon containing nanoparticles of humic substancesarehumic acid, Fulvic acid and organic compound content was in the form of GA_3, IAA and BAP amount of to 0.13 %, 0.002%, and 0.04% per unit respectively. Further, the above nano particles were subjected to FT-IR, HPTLC and HPLC and crystallization of crude plant growth hormones for confirmation.

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INTRODUCTION

Composting is the controlled microbial bio-oxidative process involving biodegradable organic matter, conducted under controlled environmental conditions. The oxidation produces a transient thermophilic stage which is followed by a period of cooling of the present degrading organic matter. Moreover, bio-oxidative metabolism of microorganisms involved ensures that the bulk of the biodegradable carbonaceous matter will be dissimilated completely to CO2 and water. Other components organic matter, such nitrogen and sulfur, will be assimilated into microbial cell mass, only to be liberated again after the cells die and degrade. Humic substances are general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight and refractory. Humic substances can be divided in three fractions such as a) humin is the fraction of humic substances that is not soluble in water at any pH; b) humic acid (HA) is the fraction of humic substances that is not soluble in water under acidic conditions (below pH = 2), but becomes soluble at greater pH values; c) fulvic acid is the fraction of humic substances that is soluble under all pH conditions (Stevenson, 1994). The oxidative metabolism of microorganisms is exothermic, and the heat produced is sufficient to increase the temperature of organic matter between 60 to 75'C over a period of 10 days. This is called "thermophilic stage" which offers a self –sanitizing mechanism by which pathogen, seeds and heat-labile microbial and plant toxins (Phytotoxins) will be destroyed (G.J.Manderson Ed., Biotechnology-Vol. VIII).

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Nanoparticle from organic composting

Dominant phases of the natural NPs include: Iron oxides/hydroxides, aluminum oxides/hydroxides, clay minerals (hydrated aluminosilicates of K, Mg, Fe etc.) and silica. Biological activities also assemble following the bottom up process with a wide range of carbon - containing NPs including humic substances, building molecules, functional enzymes, coal, and produce nanoorganisms such as bacteria, viruses, cells and their organelles. Though we usually associate air pollution with human activities such as transportation, industry, andcharcoal burning, natural events such as dust storms, volcanic eruptions and forest fires canproduce such vast quantities of nanoparticulate matter in the atmosphere that profoundly affect air quality worldwide (Buzea *et al*., 2007; Maurice and Hochella, 2008; Stefania Mura *et al.,* 2013).The spatial extent of erosion, the physico-chemical properties of the exposed organic wastages are important, especially the degree of humification and moisture content. During the latter

stages of decomposition, lignin breaksdown to humic acids, (Bozkurt 2001).

The fulvic acids are formed by decomposition of once living matter, fulvic acid contains hidden treasures of the past, in the perfect plant form, in nature's own recycling process. Especially, a nano particle of mineral, metal and trace elements complexes with fulvic acid becomes an additional bonus to the miracle of nano molecules of fulvic acid. It is hundred times smaller than living cells and also amazingly absorbable with unique ability to enhance, potentiate and increase absorption of many other nano compounds of vitamins, herbs, minerals more than 70 trace elements carrier, tinctures and food with which it is combined of unparalleled miracles nano molecules. Plants readily absorb high amounts of fulvic acid, and maintain it in their structure.

Organic compounds

Compost prepared from *Partheniumhysterophorus* (Linn.) plants, farm yard waste, maize waste and chick waste are made up of nano compounds and humic substances that contribute to the 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 with chemical, physical and biological properties (Tejada and Gonzalez, 2004).The significant growth exhibited by plants grown in compost suggests that nano compounds and humic substances provide more than just plant nutrients (nitrogen, phosphorus and potassium) for growth (Atiyeh *et al*., 2000). Further, humification is determined by costly and time consuming point sampling and wet chemistry, done by FT-IR method. Similarly, near infrared (NIR) reflectance spectroscopy offers an alternative method (McLellan *et al*., 1991). NIR-SWIR spectroscopy can be used to predict the stage of decomposition of dry, milled leaf litter (Joffre *et al.,* 1992; Gillon, *et al*., 1999), decomposing grape and cattle manure (Ben-Dor *et al*., 1997) and dried peat from a stratigraphic core (McTiernan *et al.,* 1998). Further, to study find the content of organic composting was under the fermentation samples was done by FT-IR, HPTLC and HPLC and crystallization of crude plant growth hormones.

MATERIALS AND METHODS

Study materials

A study was conducted by anaerobic treatment of compost making, the effect of phenolics and parthenin compound (Fig. 1 a & b). Different ratios of *Parthenium* plants, Cow dung (FYM), maize waste and Chick wastein the ratio (3:4:2:1) were transformed and incubate in to compost products for about 45-90º C up to 45-120 days under the anaerobic condition with complete radiation from sun light during day time and the retention time up to 45 -120 days.

Purification and extraction of humic acids from organic compost

One hundred grams of the samples were suspended in 160 ml of 0.1 N NaOH in stainless steel sealed, and agitated on a rotary shaker (130 rpm) at 30°C for 24 h. The tubes were centrifuged at 20,000 3gfor 15 min; the supernatant was acidified with 1 M HCl (final pH2.0) and kept at 4°C for 24 h to precipitate the humic acids. The humic acids were recovered by centrifugation, oven dried at 60°C, ground, and stored under nitrogen atmosphere at 218°C until they were utilized. Commercialhumic acids (technical grade) obtained from AGROS ORGANICS; CAS: 68131-04-4, New Jersey, USAwere used tocontrol. To obtain preparations free of acid-soluble iron, 200 mg of humic acids dissolved in 1 ml of anoxic 1 N NaOH was diluted with 10 ml of 1 M HCl and incubated at 30°C on a rotary shaker (90 rpm) for 24 h. The humic acids were recovered by centrifugation and suspended in distilled water. The suspension was repeatedly degassed and then flushed with N_2 with stirring, and the pH was adjusted to 7 with NaOH.

Purification and extraction of Fulvic acid

After humic acid extraction of the liquid was done initially pH of liquid approximately 2.0 and 0.1 M pyrophosphate (pH2.0) was added. Dissolution of humic acids was minimal. Pyrophosphate masks iron (III) prevents any redox reaction with polyphenols; this masking has been demonstrated by sampled dc polarographic measurements on iron (lll) pyrophosphate solutions in the presence of catechol. On acidification, prolonged standing at pH 1.7 can lead to polymerization and precipitation of fulvic acids. When this polymerization occurs to the complete retrieval of fulvic acids from the resin is impossible.

Extraction of Plant Growth Hormones

A study was carried out by CRF-SIF at Sri Ramachanra, University Porur in Tamil Nadu, and India. The nano compound was analyzed the instrumentation by win CATS Planar Chromatography Manager (HPTLC) system and identifies plant growth hormones were studied in anaerobic organic compost. Plant growth hormones were extracted from samples organic compost which has been recycled into compost. A sample of the compost was studied in anaerobically in Kalasalingam University Campus. Distilled water was first used to extract the plant growth hormones from the compost followed by the use of chilled 80% methanol.

Methanol Extraction

In order to obtain high concentration of any potential plant growth hormones present in anaerobic organic compost, the procedure described by Badr *et al*. (1971) and modified by Taylor *et al*. (2004) was adopted. Twenty five grams of sample alone was weighed into an extraction bottle and 75 ml of chilled 25, 40, 50, 60 % methanol. This process was repeated six times to obtain a final/ total volume of 450 ml of 80% methanol. One hundred and fifty mililitres of 80% methanolic slurry of sample alone was placed on a mechanical shaker for approximately for 24hrs. 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 -4°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 four times followed by bulking all the extracts were bulked and reduced to aqueous phase (48 mL) using a rotary evaporator prior to storage in a refrigerator. Further filtered materials was studied by chromatographic condition for HPTLC finger print as stationary phase should be silica gel F 254 and mobile phase is n-Hexane: Ethyl acetate (75:25) and sample scanning 485nm, the sample working concentration 25mg/ml and injected different volume of 2, 4, 6, 8 µl by using ascending mode.

RESULTS

The growths of nanotechnology applications require environmental impact and the assessment of elaborated nanomaterials to reduce environmental risks in case of their releases and possible pollution. However, fulvic acid usually carries more than 70 types of minerals and also traces elements as part of its molecular complexes. Plants readily absorbed by the roots with high amounts of fulvic acid and maintain it in their structure. The organic compounds such as Gibberellic Acid (GA3), 6-Benzyl amino purine (BAP), and Indole acetic acid (IAA) are very potent plant hormone, which regulates the growth rate of plants promoting cell elongation. Gibberellic acid stimulates the cells of germinating seeds to produce mRNA molecules that code for hydrolytic enzymes. However the auxin and cytokin nano compounds were analyzed from *Parthenium* organic compost.

Decomposing manure

Partheniumhysterophorus (Linn.) had high level of macro and micro nutrient sources of N, P K Ca, Mg and chlorophyll content and are ideally suited for composting. The highest ratio of N-2.23%, P-1.07% and K-1.92 were recorded randomly as shown in (Table-1).

Humic acid and Fulvic acid

Humic acid is very large and complex molecules extracted from organic matter have been used in many ways for plant production. The several nanomaterials and organic compounds was identified as humic acid 21%, Fulvic acid, and plant growth regulators of GA3 0.13 % (0.0348g/25ml liquid sample), IAA 0.002 % (0.0006 g/25 ml of liquid sample) and BAP 0.04 % (0.097g/25ml of liquid sample) which was found from the organic composting materials. The humification process of changing organic matter such as leaves, farm yard manure, maize waste and chick waste were humic substances by geo-microbiological mechanisms. The humification process under the anaerobic condition (45-120 days) was higher (Fig 2 a-d). As the conversion process continues, different chemicals dominate at different times. Complete conversion to humic substances will eventually occur. However, the humic acid was dissolved in water slowly for testing the percentage of humic acid (Fig 3: 1-4)

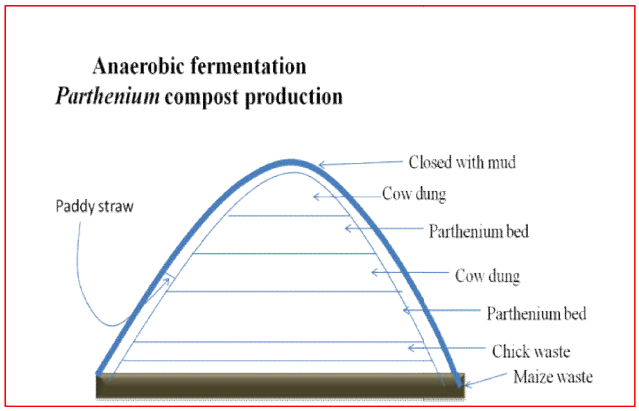


Fig 1a

Fig 1b

Fig-1a & b. The method of anaerobic fermentation of *Parthenium sp.* compost production

Table 1. The physical, chemical parameter and humic acid content in the organic compost

S.No.	Parameters			
	Physical	Chemical	Percentage of molecules	
			FYM compost	*Parthenium* compost
1	**Parthenium compost:**	Nitrogen	0.028	2.23
	Color: Brownish black	Phosphorus	0.017	1.07
	Odor: Odor less	Potassium	0.023	1.92
	Moisture: 10-25%	Manganese	0.00004	0.002
	Particle density (g/cc): 0.5-1.0	Zinc	0.0006	0.982
	pH: 6.2-6.5			
		Copper	**0.006**	**0.054**
2	**FYM compost:**	Organic carbon	10.2	16.2
		C/N ratio	5.70	10.7
	Color: Brownish black			
	Odor: Odor less	Humic acid	**0.3**	**21.0**
	Moisture: 10-25%			
	Particle density (g/cc): 0.5-1.0	Fulvic acid	0.002	5.0
	pH: 6.2-6.5			

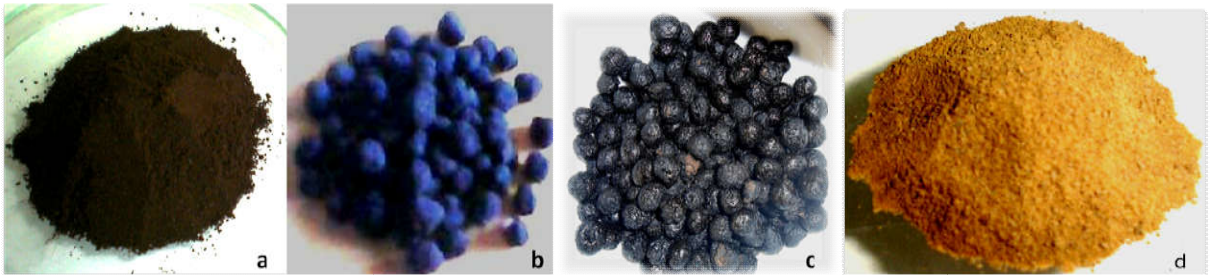


Fig-2 a, b &c: a. powdered humic acid, b & c humic acid beads handmade preparation from organic compost and d. Fulvic acid

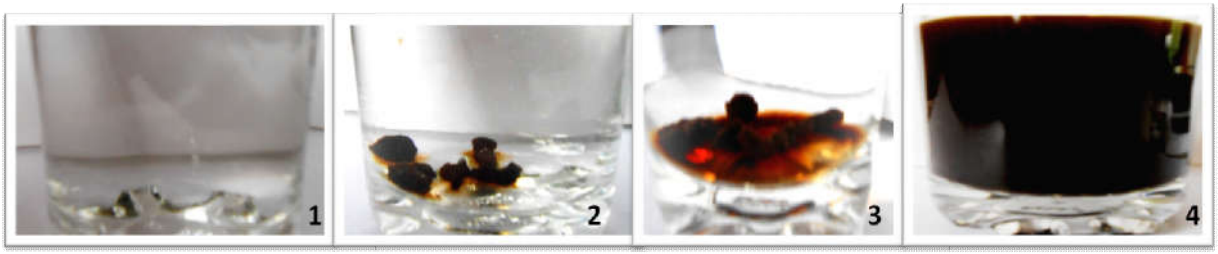


Fig-3. Humic acid product testing of dissolving nature in water (1.Control-water; 2.4-5 beads of humic acid; 3. Slowly dissolved and 4.Completely dissolved

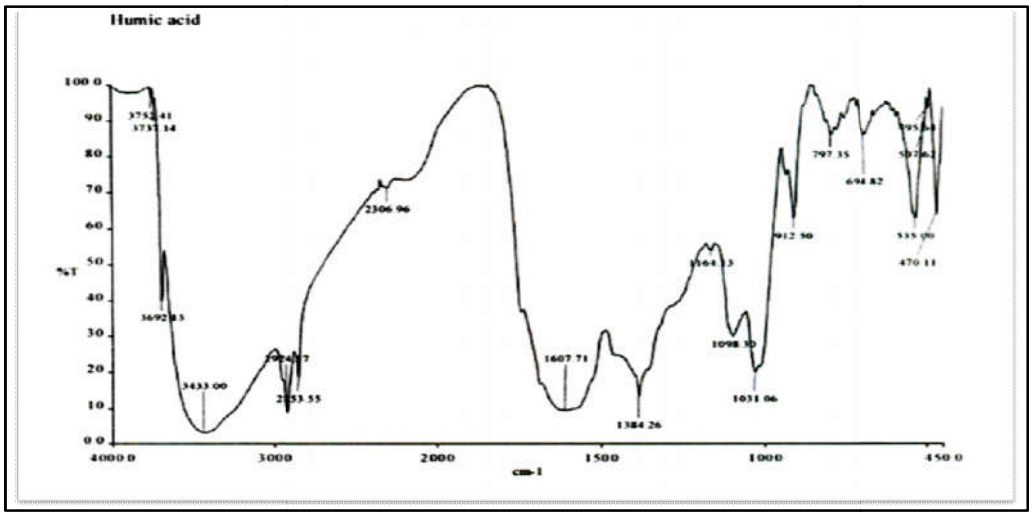


Fig-4 a. FTIR analysis of the humic acid (Control -AGROS ORGANICS; CAS: 68131-04-4, New Jersey, USA)

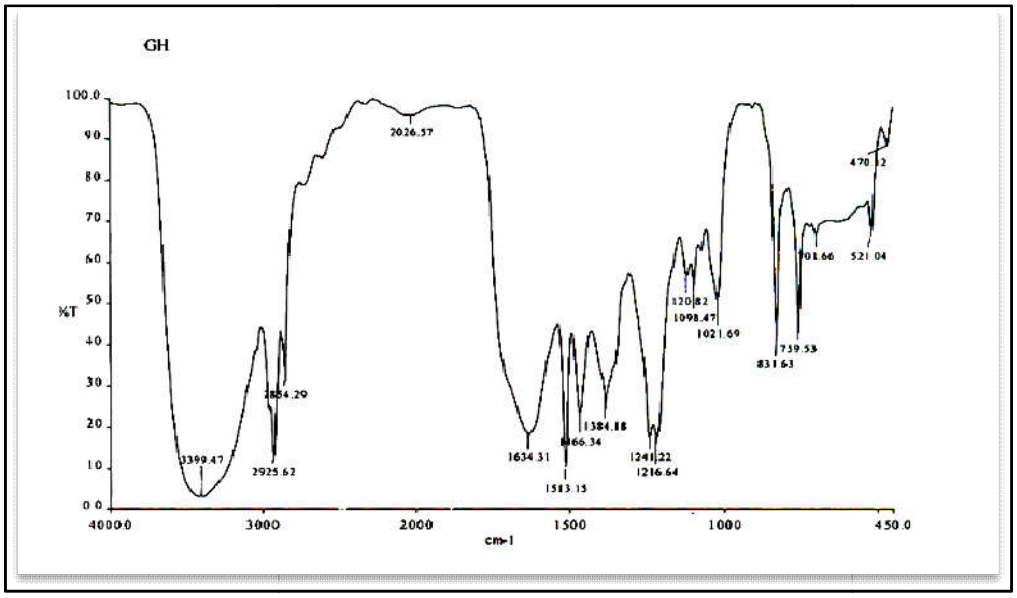


Fig-4 b. FTIR analysis of the humic acid from organic compost

Characterization of FTIR

FTIR analysis of the humic acid (Control-AGROS ORGANICS; CAS: 68131-04-4, New Jersey, USA) was carried out as follows; The organic compost from purification of humic acid sample (1 mg) was ground with 200 mg of KBr (spectroscopic grade) in a mortar before pressed into 10 mm diameter disks under 6 tons of pressure. FTIR spectra were obtained on FT–IR 8300, Shidmadzu spectrometer. The analysis conditions used were 16 scans at a resolution of 4 cm-1 measured between 400 and4,000 cm-1. From the spectrum obtained (Fig. 3), in control of humic acid showed a broad band at 2,433.00–2,853.55cm-1 which correspondsto O–H stretching that also overlapped with N–H stretching of a secondary amideat 3692.43 cm-1. Secondary amides are associated through H-bonding to formdimers or polymers (Trans configuration) resulting in the replacement of free N–H stretching band. The weak band at3692.43 cm-1 may be due to an overtone of the band at 1607.71cm-1. In contrast, our product of the humic acid from *Parthenium* sp. and other wastages has got typical result which was found for the first time within 40-90 days incubation (Fig. 4). The humic acid showed that a broad band at 2,854.29 – 2925.62 cm-1 to measured between 400 and 4,000 cm-1. S-O stretching, aromatic groups may be regarded as insignificant between 759.53 – 831.63 cm-1 and 1021.69 – 1098.47 cm-1 get C-O stretching of polysaccharide-like substances, Si-O of silicate impurities, 1216.64 – 1241.22 cm-1 shows C-O stretching and OH deformation of COOH, 1384.18 – 1466.34 cm-1 OH deformation and C-O stretching of phenolic OH, C-H deformation of CH2 and CH3 groups, COO^- antisymmetric stretching and C=C stretching within the ring was predominantly found. However, 1516.15 cm-1 shows COO^- symmetric stretching, N-H bending, C=N stretching (amide II bond) and 1634.31 cm-1 have C=O stretching of amide groups (amide 1 band), quinone C=O and/or C=O of H bonded conjugated ketones was recorded.

Methanol extraction

Thin layer of chromatography (TLC) and high performance of thin layer chromatography (HPTLC) – are now also called planar chromatography – are, like all chromatographic techniques, based on a multistage distribution process.

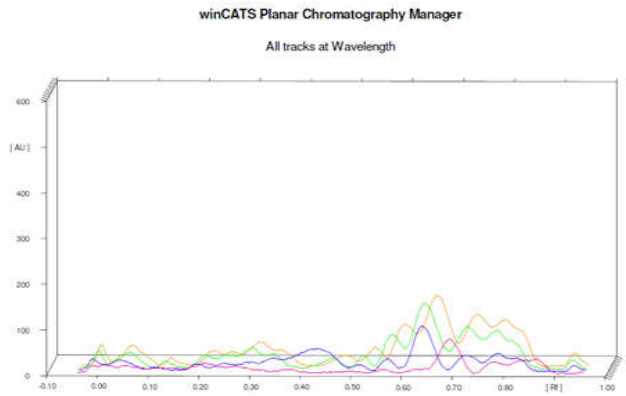


Fig 5. The purification of plant growth regulator for four different types of methanol fraction (25, 40, 50 & 60%) from the organic compost and run on the HPTLC

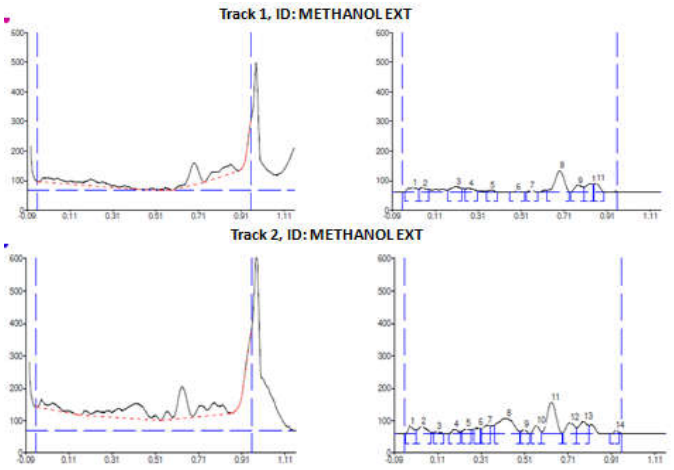


Fig 6 a. The purification of plant growth regulator for 25 and 40 % methanol extract track – 1&2 results on the HPTLC from the organic compost

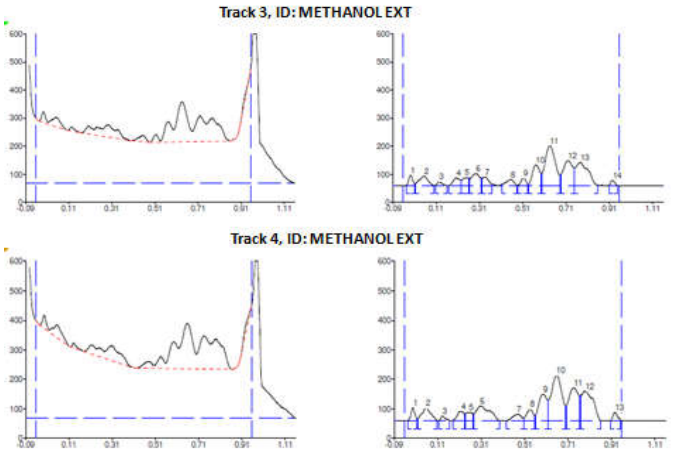


Fig 6 c. The purification of plant growth regulator from the organic compost for 50 and 60 % methanol extract track -3 & 4 results on the HPTLC

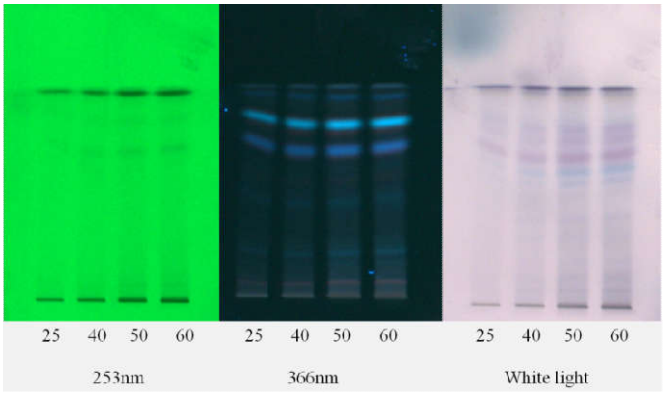


Fig 7. The purification of plant growth regulator from the organic compost for 25, 40, 50 and 60 % methanol extract track 1-4 run on the TLC plate and different wave length Light were exposed

This process involves: a suitable adsorbent (the stationary phase), solvents or solvent mixtures (the mobile phase or eluent), and the sample molecules to run on TLC plate (Fig.). The study was conducted by using four different types of

methanol extraction such as 25, 40, 50 and 60 % which were used as organic compost. The chromatographic condition for HPTLC finger print study was conducted and different types of nano compound expresses very clearly in the 253nm as green light, 366nm UV transilluminator and compared to white light. The different extraction to run on the HPTLC system (Fig-5) gives similar results of nano compound which was found in 40, 50 and 60 % and little contrast in 25 % methanol extraction (Fig a,b,c & d). Although, organic compost have lot of 1-14 type of organic compound was in HPTLC analysis, and all of them unknown are identified. A further study was conducted specifically plant growth hormones using HPLC as a control GA3, BAP, IAA, IBA and NAA.

Extraction of plant growth regulator

Extraction, purification and quantitative determination of free and bound GA3, IAA, and BAP in all of them contain organic compost were conducted with little modifications in the methods of Prakash and Prathapasenan (1990), Zieslin and Geller (1983), Ames *et al.* (1979), Beardsell and Cohen (1975) and Van Staden and Nicholson (1989). The extraction and purification procedures are shown in Fig. 8a. In the testing procedure of recovery, authentic IAA, GA3, BAP 24D and IBA standards were used and each was 1mg with 100 ml of methanol: chloroform:2N ammonium hydroxide (12:5:3 v/v/v).

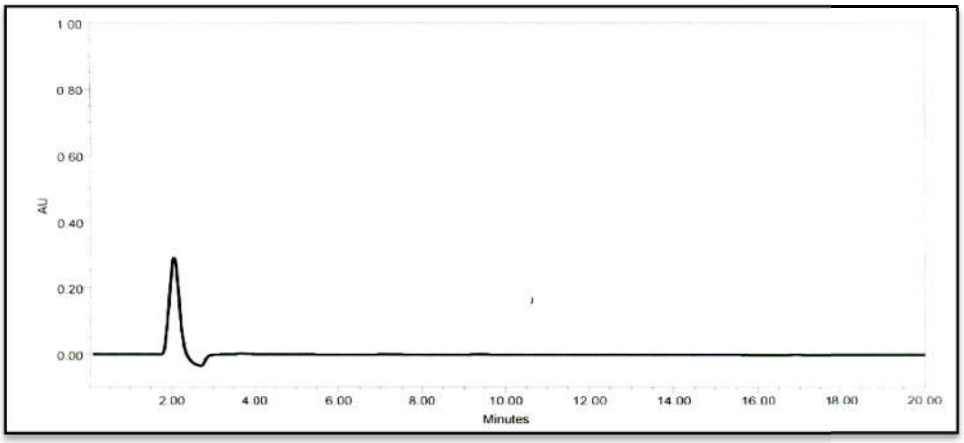


Fig 8a. The control of HPLC water alone on HPLC results

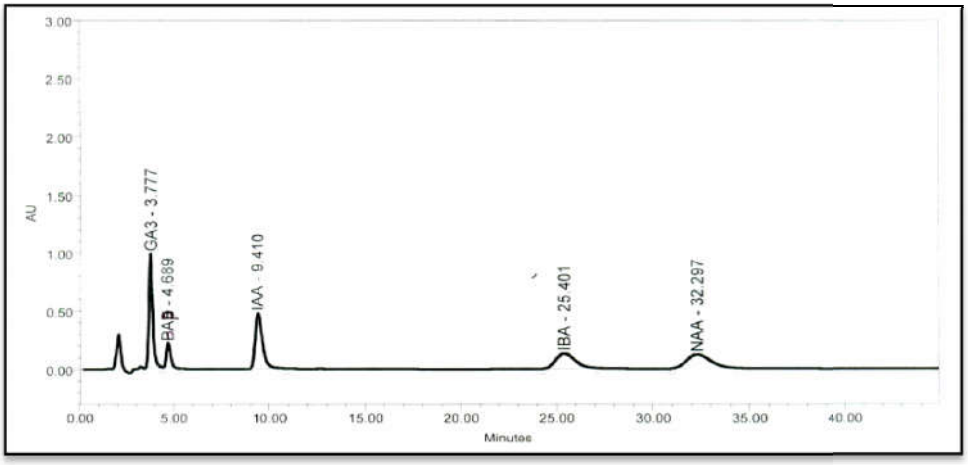


Fig 8b. The control of HPLC water alone on HPLC results

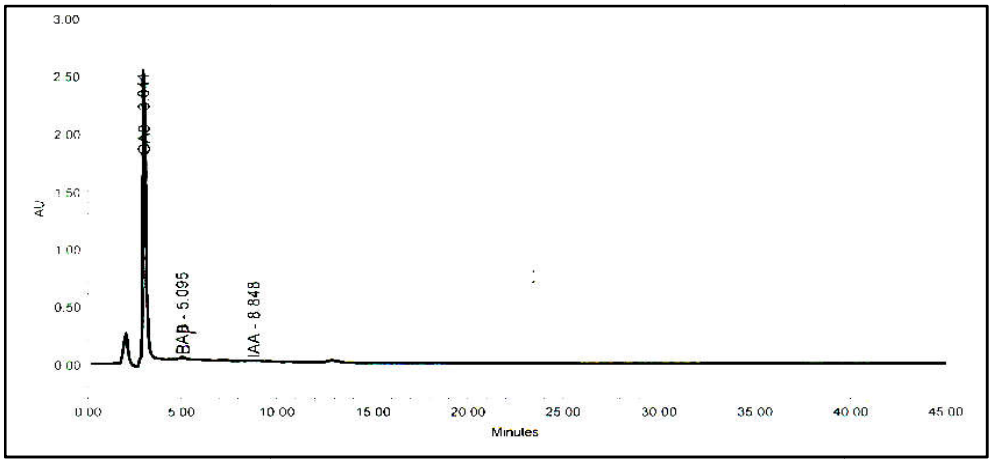


Fig 8c. The control of HPLC water alone on HPLC results

IAA, GA3, BAP, 24D and IBA loses during the extraction and purification steps were determined by passing known amounts of standard synthetic IAA, GA3, BAP 24D and IBA samples through the Fig. 8bprocedure. Spectrophotometric techniques were applied to determine the amounts of IAA, GA3, and BAP. Average recovery of IAA, GA3, and BAP are displayed in percentage which is calculated by total amount of samples (Table-2) and Fig 8c.

Several methods are involved in the purification of plant growth regulator, and little modified crude crystallization of plant growth regulator from our organic compost sample has been done (Fig 9 a, b &c).

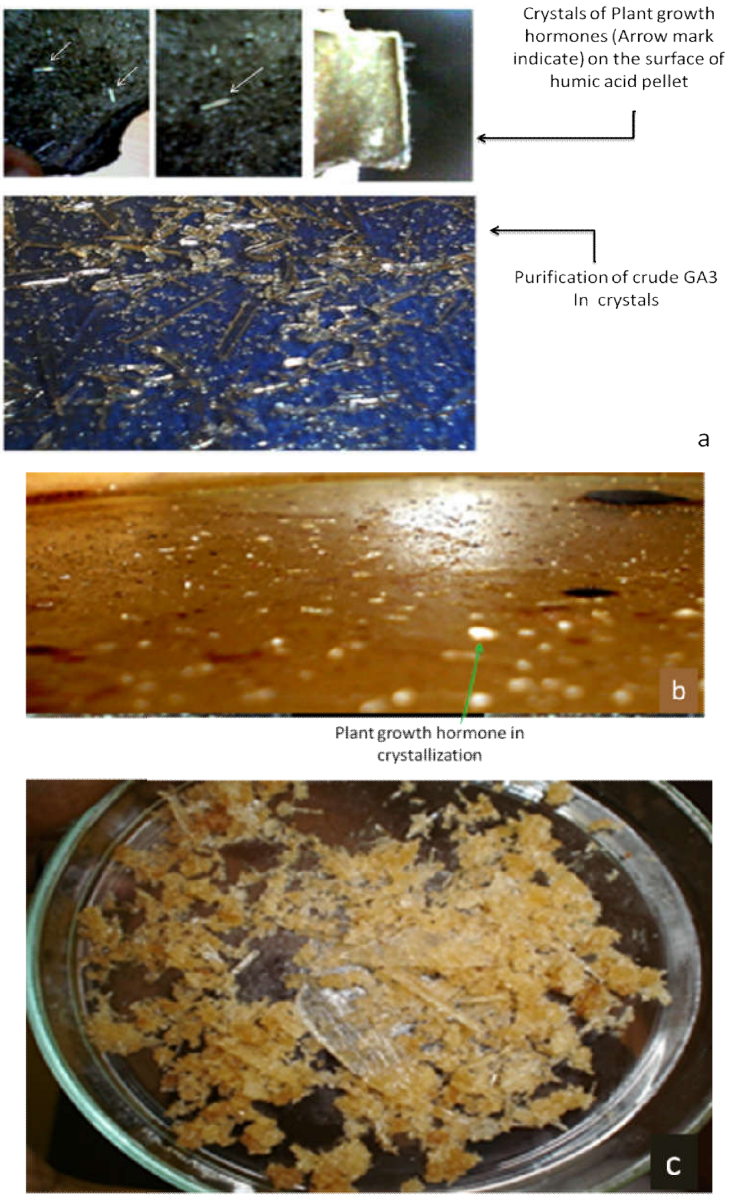
DISCUSSION

Since the nano molecules involved in plant growth regulation and development have received increasing attention over the

Table 2. The plant growth regulator and sample of organic compost from phytohormone analysis on HPLC and percentage of yield from sample

S.No	Growth Hormones	Amount (gm)/25ml of sample	Percentage (% W/V)
0	Control (HPLC water alone)	-	-
1	GA3 (Gibberlic acid)	**0.0348**	**0.13**
2	BAP (6-Benzyl amino purine)	**0.0097**	**0.04**
3	IAA (6-Indole acetic acid)	**0.0006**	**0.002**
4	IBA (Indole butric acid)	-	-
5	NAA (Napthaline acetic acid)	-	-

Purification of plant growth regulator



Fig(9a & b, c). The purification and crystallization GA3 and crude plant growth hormones from organic compost

past decade from the organic compost, large number of purification nano compounds of humic and fulvic and organic compounds of indoles, cytokineand humatein organic compost extracts for the first time under the dry anaerobic organic *Parthenium* composting (Buzea *et al*., 2007). In practice it is often necessary to use more than one procedure to achieve an adequate degree of sample purity prior to analysis. The growth of nanotechnology applications requires environmental impact assessment of elaborated nanomaterials to reduce environmental risks in case of their releases and possible pollution. Fulvic acid is a part of the humic structure in rich composting and it is an organic acid created in extremely small amounts by the action of millions of beneficial microbes, working on decaying plant matter in soil environment with adequate oxygen. It is of low molecular weight and is biologically very active. However, fulvic acid usually carries more than 70 types of minerals and also trace elements as part of its molecular complexes. Plants readily absorbed by the roots high amounts of fulvic acid and maintain it in their structure. The organic compounds such as Gibberellic Acid (GA3), 6-Benzyl amino purine (BAP), and Indole acetic acid (IAA) is a very potent plant hormone, which regulates the growth rate of plants promoting cell elongation. Gibberellic acid stimulates the cells of germinating seeds to produce mRNA molecules that code for hydrolytic enzymes. However the auxin and cytokin nano compounds were analyzed from *Parthenium* organic compost.

A group of workers also proposed that the humic acid and fulvic acid present in the nano compounds 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 organic compost. 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 of 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). 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 the 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 thanlypophilic. 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). By the end of 45-120-days period, significant differences were found in the *Parthenium* compost content when compared to FYM Compost. Table 1 shows the macro and micro nutrient content of *Parthenium* and FYM compost. After45-120 days was decomposing there more significantly than the differences in the *Parthenium* and FYM contents.

Conclusion

The *Parthenium* organic composting results showed the results of C-containing nanomaterials in the form of organic colloids such as humic acid and humic acid and organic compounds of Gibberlic acid (GA3), 6-Indole Acetic acid (IAA), and 6-Benzyl amino purine (BAP) in the organic compost. The contents were obtained by humic acid 21%, Fulvic acid, and plant growth regulators of GA3 0.13 % (0.0348g/25ml liquid sample), IAA 0.002 % (0.0006 g/25 ml of liquid sample) and BAP 0.04 % (0.097g/25ml of liquid sample) based on the study was confirmed by FT-IR, HPTLC, and HPLC results.

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