Development of organic manure in the organic cultivation of Tomato: SEM-EDS & GC-MS analysis

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Abstract: In this study, coir pith based organic manure was applied in the organic cultivation of *Solanum lycopersicum* L. (Tomato). Addition of this organic manure, effect the elemental composition and relative distribution of nutrients in plant fruits was evaluated. In a greenhouse, organic tomatoes were cultivated adding the minimum particle size of organic manure and the results were compared with the control. SEM-EDS and GC-MS analysis were studied in the obtained plant fruits. The obtained fruits were analyzed for the presence of Phytochemicals and elemental composition using GC-MS and SEM-EDS analysis. Results revealed that the minimum particle size (0.01-0.1mm) of organic manure applied plant fruits have high nutrient status, as well as induce the elemental composition, relative distribution of nutrients and the presence of more number of volatile compounds than the control. The addition of minimum particle size of organic manure to plants were positively influenced their development. Based on the nutrient status, the organic plant fruits showed significant effect than control. It revealed that the addition of minimum particle size of organic manure positively influences the health benefits or food security of the produced fruits.

Keywords: Coir pith, Cyanobacteria, Cyanopith, Jiwamrita, Manure, Tomato

1. INTRODUCTION

Coir pith is a lignocellulosic waste which is dumped as a huge pile on the roadside also contains high lignin content and slow to degrade in a natural environment (Bhat and Narayan, 2003). Disposal of coir pith has become a major problem for the industries. However, at the same time coir pith is used for many commercial applications (Abhijith and Prashanth, 2003). Coir pith based cyanobacterial biofertilizer could be an effective other choice for chemical fertilizer that make plant grow better (Christopher *et al.* 2007). Application of coir pith manure effectively improves the physico- chemical properties of the soil and induces the crop yield (Abesh and Anita Das, 2010). The organic amendments are used to improve soil productivity and to provide a source of nutrients in organic agriculture practices (Karimi *et al.* 2017).

Cyanobacteria are despicable to maintain with high growth potential and have an ability to degrade the coir pith to be used as biofertilizer and are also effective in reducing the pollution. Microbial degradation of coir pith is considered as a safe, valuable and environmentally friendly process. The lignocellulosic material must be converted into simpler compounds by composting process for easy uptake by the plant. Cyanopith is an organic biofertilizer prepared by the degradation of coir pith using fresh water Cyanobacterium, *Oscillatoria annae* (Malliga *et al.* 2012). Coir pith degradation by Cyanobacterium is a partial degradation and also application of coir pith based cyanobacterial biofertilizer (cyanopith) to the soil takes more time to degrade by soil microbes. Preparation of minimum particle sizes can be easily degraded by soil microbes which can promote easy uptake by plants hence, increasing the productivity of crops (Jenny and Malliga, 2016).

Jiwamrita is a plant growth promoting substance containing beneficial microorganisms (Vanaja *et al.* 2009) furthermore improving the physicochemical and biological properties of soil and also, the efficiency of applied manure (Manjunatha *et al*. 2009). They also confirmed that the potential of Jiwamrita is to supply materials and to act as food support for beneficial microbes. Tomatoes are a juicy berry fruit of the nightshade family (*Solanaceae*) which provide good quantities of vitamin A and C so as to most versatile vegetables in the world because of its highly nutritive value, taste, color and its diversified use (Angole, 2010). Hence, the aim of this work was to determine the effects of the application of the minimum particle size of organic manure on organic tomato cultivation in the greenhouse. In particular, the effects of this organic matter addition on fruit production and nutritive value were assessed. In addition to profiling, the presence of mixtures of metabolites in the organic tomato fruits using GCMS and SEM-EDS.

2. MATERIALS AND METHODS

2.1. Development of organic manure

The fresh water Cyanobacterium, Oscillatoria annae was obtained from the germ plasm of National Facility for Marine Cyanobacteria (NFMC), Bharathidasan University, Tiruchirappalli, Tamil nadu, India and grown in BG11 medium **(**Rippka *et al.* 1979), under white fluorescent light (10/14 hrs. L/D cycle) of 1,500 Lux at 25 ±2º C. Then, the culture was grown with coir pith for the mass production of cyanopith under field conditions. The coir pith based cyanobacterial product was known as cyanopith and this was used as a basal fertilizer (Malliga *et al.* 2012). The cyanopith fertilizer was ground and sieved into three different particle sizes and then the samples were enriched with jiwamrita for the further degradation process (Jenny and Malliga, 2014).

2.2. Organic cultivation of Tomato

Tomato seeds were collected from the organic farm Pudukkotai District, Tamilnadu, India. Seeds were sown in two pots and were irrigated twice a day. After three weeks, the nursery was grown upto 20 cm and equal length of plants were selected for transplanting. There were 9 sets of pots in each treatment and without application of organic manure was considered as a control. Single seedling of around 3 weeks old was planted per pot and was irrigated twice a day. In this study, the 20g of optimized concentration of minimum particle size of organic manure applied *S. lycopersicum* plant fruits were used and compared with control plant fruits (Jenny and Malliga, 2016).

2.3. SEM-EDS & GC-MS analysis

SEM - EDS was used to identify the chemical composition and relative distribution of macro and micro nutrients. Minimum particle sized organic manure 20g applied tomato plant fruits were harvested and washed with distilled water for surface sterilization, then the fruits were cut into small pieces with sterile knife and kept in a sterile tray covered with a net for drying. The dried powder samples were coated with gold, through a supply of nitrogen gas and subjected to SEM-EDS. In GC-MS, the chemical properties between different molecules in a mixture will separate the molecule as the sample travels the column and the mass spectrometer break each molecule into an ionized fragment and detect them using their mass to charge ratio (Srimah, 2009).

3. RESULT AND DISCUSSION

3.1. SEM-EDS analysis

The elemental composition and relative distribution of nutrients in *Solanum lycopersicum* (tomato) fruits were analyzed using SEM-EDS studies. The EDS spectra showed an elemental composition and relative distribution of nutrients was increased in the organic sample than the control sample (Fig. 1). Results revealed that the minimum particle size of organic manure applied plant fruits exhibited the significant variations and the presence of elements in the following order: K>Cl> P> Mg> Ca> S> Zn> Mn> Cu> Fe and in control plant fruits K> P> Cl> Mg> S> Cu> Ca (Table-1).

Scanning electron microscopy (SEM) coupled with energy-dispersive X-ray analysis (EDS) is an effective method that can yield both qualitative identification and quantitative elemental information, based on the characteristic X-radiation emitted from samples, thus also allowing the direct observation, comparison and characterization of different materials (Goldstein *et al.* 2003). *Azospirillum*-inoculated and control Strawberry plants had similar elemental quantities; however, in bacteria-inoculated roots, P were significantly increased, while Cu content decreased (Guerrero *et al.* 2014). The *A. hypogea* treated with 2% *H. musciformis* (Seaweed Liquid Fertilizer) results obtained from the scanning electron microscopic image analysis of different chemical elements, namely N, P, K, Ca, S, Na, Mg, Mn, Zn and Fe were observed in the cell wall of leaf of 2% SLF treated and control of *A. hypogea* (Ganapathy and Sivakumar, 2014). The EDS analysis of *V. mungo* obtained different chemical elements present in the cell wall of 2% SLF treated leaf and control (Ganapathy and Sivakumar, 2013).The qualitative and quantitative compositional analysis of *S. wightii* provides the localized distribution of chemical elements of leaf by energy dispersive X-ray Microanalysis (Sundari and Selvaraj, 2009). The SEM-EDS method was used to examine morphological changes, the relative distribution of macro- and micronutrients and chemical composition on the surface of REC3-inoculated plants (Guerrero *et al.* 2012). The sodium (Na) appeared to have a useful impact on the development of strawberry cv. Korona under saline anxiety (Saied *et al.* 2005), which could be identified with the substitution of K for Na, which helps in osmoregulation to keep up the water substance of plant tissues and at last increment of fresh weight (Turhan and Eris, 2004). Esitken *et al.* (2010) showed a comparative addition in P take-up in strawberry plants vaccinated with others PGPB strains: *Pseudomonas* BA-8, *Bacillus* OSU-142 and *Bacillus* M-3.

3.2. GC-MS Analysis

Gas Chromatography Mass Spectroscopy is widely used to profile and analyze the mixtures of complicated metabolites like organic acids, sugars, amino acids, lipolytic compounds and phosphorylate intermediates. In this study, the 20g of optimized concentration of minimum particle size of organic manure applied *S. lycopersicum* plant fruits were used and compared with control plant fruits. Results revealed that the application of optimized concentration of 20 g of the minimum particle size of organic manure applied tomato fruits were richer in metabolic compounds than the control fruits (Fig. 2). Nevertheless, the metabolic compounds were represented in Table-2 and these include free acids, sugars, aldehydes, ketones, acetic acid, propanic acid, benzoic acid, hexonic acid and glucose. The volatile compounds such as nitrogen, oxygen, sulfur, phenols, free acids, heterocyclic compounds aldehydes, ketones, esters, alcohols, hydrocarbons, ethers and lactones were detected in tomato using GC-MS (Rastogi and Davies, 1991). Halmja *et al.* (2007) reported *Solanum lycopersicum*, *S. tuberosum* and *S. Melongena* were rich in vitamins and phenolic compounds which were examined by GC-MS technique. Moreover, higher quantities of volatile compounds such as methyl-butanol were found in tomato samples (Queralt *et al.* 2013).

GC-MS analysis used to find the polysaccharides present in pericarp discs of tomato fruit (Greve and Labavitch, 1991). The seed oil of tomato involved linoleic acid, palmitic acid and oleic acid. So, it was the best source to get the most significant fatty acids like oleic acid and linoleic acid (Botinestean *et al.* 2012). GC-MS used to analyze the volatile compounds found in tomato such as β-carotene, chlorophyll, lycopene, α-tomatine, dehydro tomatine tetra saccharide, glycol alkaloids and the anti-cholinergic alkaloids, atropine are the main economical food source with low-fat in tomato used to obtain energy, nutrients and also provides bioactive secondary metabolites, which either have harmful or helpful effects of diet (Friedman *et al.* 2004). Oms-Oliub *et al.* (2011) reported the metabolic profiling characterization during preharvest development, ripening and postharvest shelf-life of tomato fruit. Most compound level in groups, showing either increasing (e.g., maleic and aspartic acid) or decreasing levels (e.g., valine and malic acid) with fruit development and with some compounds. The major hexoses, glucose, fructose and cell wall components such as galacturonic acid and the amino acids such as aspartic, glutamic acid and methionine were increased during the ripening stage of tomato fruits. The most abundant volatile compounds in tomato fruits were derived from lipids such as linoleic and linolenic acids as: hexanal, trans-2-hexenal, cis-3-hexenol (Farneti *et al.* 2012).

The major organic acids of the fruit were found different from the leaf, although citrate and malate and D-iso ascorbate were present at high levels, the level of succinate was lower in the fruit. The levels of galacturonic acid, gluconate and iso citrate are considerably higher in the fruit than in the leaf. Several metabolites such as chlorogenate and nicotinate were detected in tomato fruit (Nicolas *et al.* 2005). GC-MS was used to identify both polar and volatile metabolites for the various stages of fruit development and ripening, such as mannose, citramalic, gluconic and keto-1-gulonic acids also, were shown to be strongly correlated to the final postharvest (Luengwilai *et al.* 2012).

4. CONCLUSION

The present study confirmed the expectation that the minimum particle size of organic manure can be applied in the organic cultivation of tomato. The organic manure addition was biostimulating to the plants, influencing development positively, in addition to increasing fruit production and the obtained fruits were subjected to SEM-EDS and GC-MS analysis. However, the minimum particle size of organic manure applied plant fruits exhibited the significant variations and the presence of more no. of elements than control plant fruits. GC-MS results revealed that the application of optimized concentration of 20g of the minimum particle size of organic manure applied tomato fruits were enriched in metabolic compounds than the control fruits. Hence, this study concluded that the minimum particle size of organic manure has high nutrient status, as well as furnish good yield of tomato *(Solanum lycopersicum* L.) as compared to that of control.

ACKNOWLEDGEMENT

The author is grateful to Model Organic Farm (MOF), Bharathidasan University, Tiruchirappalli, Tamilnadu for the facility and completion of this work.

REFERENCES

[1] Abesh Reghuvaran, Anita Das Ravindranath (2010) Efficacy of biodegraded coir pith for cultivation of medicinal plants. *J. of Scientific and Industrial Research.* 69: 554- 559.

[2] Abhijith Bhat D, Prashanth N (2003) Chromatographic Analysis of Phenolics and study of Klason lignin biodegraded coir pith using *Pleurotus sajor-caju.* B.Sc., Dissertation submitted to University of Kerala, Alappuzha.

[3] Angole (2010) A field Study of three organic manure on yield of tomatoes. *In*: Bradt Travel Guides, pp. 81-83.

[4] Bhat AD, Narayan P (2003) Chromatographic Analysis of phenolics and study of Klason lignin biodegraded coir pith using "*Pleurotur sajorcaju*" [dissertation], University of Kerala, Alappuzha.

[5] Botinestean C, Hădărugă NG, Hădărugă DI, Jianu I (2012) Fatty Acids Composition by Gas Chromatography – Mass Spectrometry (GC-MS) and most important physical, chemicals parameters of Tomato Seed Oil. *Journal of Agro alimentary Processes and Technologies.* 18(1): 89-94.

[6] Christopher PA, Viswajith V, Prabha S, Sundhar K, Malliga P (2007). Effect of coir pith based cyanobacterial basal and foliar biofertilizer on *Baseela Rubra* L. *Acta Agriculturae Slovenica.* 89(1): 59-63.

[7] Esitken A, Yildiz HE, Ercisli S, Donmez MF, Turan M, Gunes A (2010) Effects of plant growth promoting bacteria (PGPB) on yield, growth and nutrient contents of organically grown strawberry. *Scientia Horticulturae*. 124: 62–66.

[8] Farneti B, Cristescu SM, Costa G, Harren FJM, Woltering EJ (2012) Rapid Tomato Volatile Profiling by Using Proton-Transfer Reaction Mass Spectrometry (PTR-MS). *J. Food Sci.* 77: 551-559.

[9] Friedman E, Carrari F, Liu YS, Fernie AR, Zamir D (2004) Zooming in on a quantitative trout for tomato yield using inter specific introgressions. *Science.* 305: 1786–1789.

[10] Ganapathy S, Sivakumar K (2014) Influence of seaweed extract as an organic fertilizer on the growth and yield of *Arachis hypogea* L. and their elemental composition using SEM–Energy Dispersive Spectroscopic analysis. *Asian Pac. J. Reproduction.* 3(1): 18-22.

[11] Ganapathy selvam G, Sivakumar K (2013) Effect of foliar spray from Seaweed Liquid Fertilizer of *Ulva reticulata* (Forsk.) on *Vigna mungo* L. and their elemental composition using SEM–Energy Dispersive Spectroscopic analysis. *Asian Pac. J. Reproduction.* 2(2): 119-125.

[12] Goldstein J, Newbury DE, Joy DC, Lyman CE, Echlin P, Lifshin E, Sawyer L, Michael JR (2003) In: Scanning Electron Microscopy and X-ray Micro analysis. Third Edition. pp. 689.

[13] Greve CL, Labavitch MJ (1991) Turnover of cell wall polysaccharides in elongating pea stems segments. *Plant physiology.* 97: 1456-1461.

[14] Guerrero-Molina MF, Lovaisa NC, Salazar SM, Díaz-Ricci JC, Pedraza RO (2014) Elemental composition of strawberry plants inoculated with the plant growth-promoting bacterium *Azospirillum brasilense* REC3, assessed with scanning electron microscopy and energy dispersive X-ray analysis. Plant Biology. 16: 726-731.

[15] Guerrero-Molina, MF, Winik BC, Pedraza RO (2012) More than rhizosphere colonization of strawberry plants by *Azospirillum brasilense*. Applied Soil Ecology. 61: 205–212

[16] Halmja A, Veher M, Gorbatsova T, Kalijur M (2007). Proceedings of the Estonian Academy of Scienes. Chemistry. 56(4): 172-186.

[17] Jenny S, Malliga P (2014) Influence of organic manure on morphological and yield attributes of tomato plants. International Journal of Innovative Research in Science & Engineering. 2 (8): 1-6.

[18] Jenny S, Malliga P (2016) Assessment of different concentrations of organic manure on the growth and yield of S*olanum lycopersicum* L. (tomato). International Journal of *Innovative Research in Science, Engineering and Technology.* 5(3): 3722-3731.

[19] Karimi H, Mokhtari M, Salehi F et al (2017) Changes in microbial pathogen dynamics during vermicomposting mixture of cow manure–organic solid waste and cow manure–sewage sludge. *Int J Recycl Org Waste Agric* 6:57–61.

[20] Luengwilai K, Saltveit M, Beckles DM (2012) Metabolite content of harvested Micro-Tom tomato (*Solanum lycopersicum* L.) fruit is altered by chilling and protective heat-shock treatments as shown by GC-MS metabolic profiling. *Postharvest Biology and Technology.* 63: 116-22.

[21] Malliga P, Anita Das Ravindranath, Sarma US (2012) Hand book of Preparation and Application of Coir Pith Based Cyanobacterial Biofertilizer (Cyanopith and Cyanospray) for field, Priya publication.19 -20.

[22] Manjunatha GS, Upperi SN, Pujari BT, Yeledahalli NA, Kuligod VB (2009) Effect of farm yard manure treated with jeevamrutha on yield attributes, yield and economics of sunflower (*Helianthus annuus* L.). *Karnataka J. Agric. Sci.* 22(1): 198-199.

[23] Nicolas S, Zamir D, Fernie AR (2005). Metabolic profiling of leaves and fruit of wild species tomato: a survey of the *Solanum lycopersicum* complex. *J. Exp. Bot.* 56(410): 297-307.

[24] Oms-Oliub G, Hertog MLATM, (2011) Metabolic characterization of tomato fruit during preharvest development, ripening and postharvest shelf-life. *Postharvest Biology and Technology.* 62: 7-16.

[25] Queralt VA, Bendini A, Tesini F, Valli E, Maria R, Raventos L, Toschi GT (2013) Identification of various chemical constituents in tomato species using GC-MS technique. *J. Agri. & Food chem.* 61(5): 1044-1050.

[26] Rastogi R, Davies PJ (1991) Polyamine Metabolism in Ripening Tomato Fruit: II. Polyamine Metabolism and Synthesis in Relation to Enhanced Putrescine Content and Storage Life of a/c Tomato Fruit. *Plant Physiol.* 95(1): 41-45.

[27] Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.*111:1-61.

[28] Saied AS, Keutgen AJ, Noga G (2005) The influence of NaCl salinity on growth, yield and fruit quality of strawberry cvs. ‘Elsanta’ and ‘Korona’. *Scientia Horticulturae*.103:289–303.

[29] Srimah PK (2009) Towards valorization of *Prosopis juliflora* as an alternative to the declining wood resource in Kenya. Ph. D., Dissertation. Universite Henri.

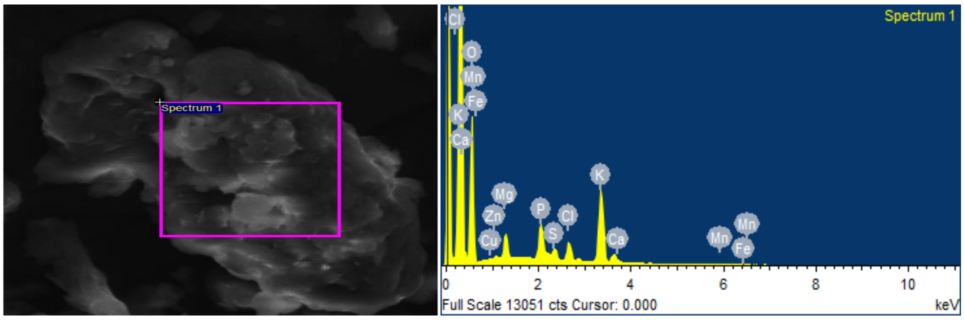
[30] Sundari K, Selvaraj R (2009) Electron microscopic study and X-Ray microanalysis of *Sargassum* species. *Seaweed Res. Utiln.* 31(1&2): 85-94.

[31] Turhan E, Eris A (2004) Effects of sodium chloride applications and different growth media on ionic composition in strawberry plants. *Journal of Plant Nutrition*. 27: 1653–1665.

[32] Vanaja R, Srikanthamurthy HS, Ningappa K, Shivakumar K, Nagaraju B (2009) Sustainable Agricultural Practices, Green Foundation, Bangalore, 52.

Fig. 1 Effect of organic manure on the elements of *S. lycopersicum* fruits using SEM-EDS analysis

a. Control sample



b. Organic sample

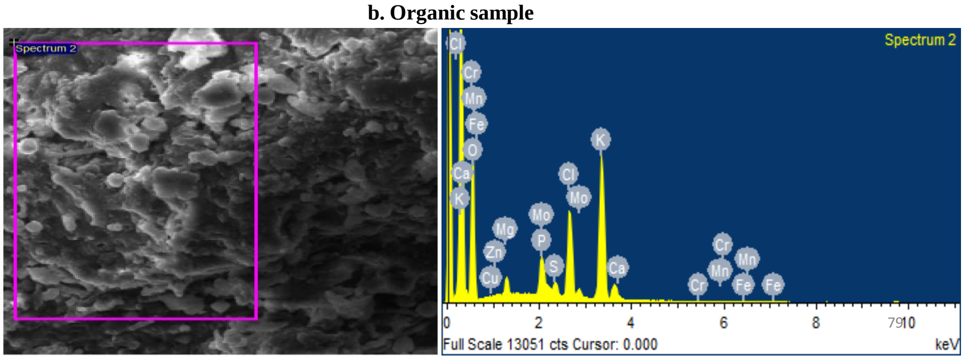


Table 1 SEM-EDS analysis of *S. lycopersicum* fruits

Elements (%)	S	Cl	K	P	Mg	Ca	Mn	Fe	Cu	Zn
Control	1.38	3.21	14.51	3.36	1.87	0.25	-	-	0.37	-
Test	1.16	9.45	18.40	4.89	4.01	0.35	0.05	0.02	0.04	0.12

Fig. 2: Effect of organic manure on the compounds of *S. lycopersicum* fruits using GC-MS analysis

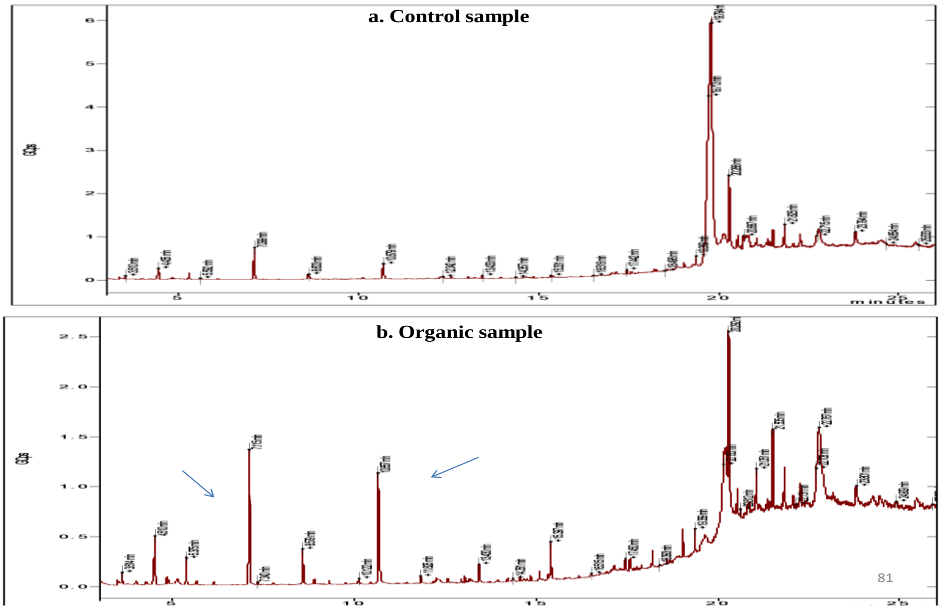


Table 2 GC-MS analysis of *S. lycopersicum* fruits

Compounds	Control	Test
Propane 1,1, diethoxy, 2methyl	+	+
Propanedioc acid oxo ethyl methyl	+	-
2 fluoropropane	-	+
1,2 : 5,6- Dianhydrogalactitol	+	+
1- Pentanol	-	+
1-Propanol, 3 (octadecyloxy)	+	+
Propane, 1,1,3, triethoxy	+	+
Acetic acid methoxy- anhydride	-	+
1-[3(4-Bromophenyl)-2-thiouredo]-1-deoxy-h-d-glucopyranose 2,3,4,6,-tetra aceta	+	+
Propanoic acid	-	+
Butanoic acid, 3 methyl	+	+
2,4, Decadienal (E,E)	+	+
Hexanoic acid	+	+
Pentanoic acid, 3 methyl	+	+
2(3H)-furanone, dihydro-3-hydroxyl-4, 4-dimethyl	+	+
2-Undecanone, 6, 10-dimethyl	+	+
Eicosanoic acid, 15 methyl	+	+
Hexadecanoic acid, 15 methyl	+	-
n-Hexodecanoic acid	+	-
Diethylphthalate	-	+
2(sec-butoxycarbonyl) benzoic acid	-	+
2 (isobutoxycarbonyl) benzoic acid	+	+
9, 12, octadecadienal	-	+
11, 14,Eicosodienoic acid, methyl ester	+	+
3,oxo- 4 phylbutyronitrile	-	+
Benzeneacetic acid	-	+
Propanedioic acid	+	+
L- Glucose	-	+
2, 4: 3, 5-Dimethelene acid	-	+