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**Original article**

# Volatile metabolites fingerprinting to discriminate the major post harvest diseases of mango caused by *Colletotrichum gloeosporioides* Penz. and *Lasiodiplodia theobromae* Pat.

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**Abstract**

Volatile metabolites emitted from mango fruits artificially inoculated with two toxigenic fungi isolated from infected mangoes were profiled using gas chromatography/mass spectrometry. A noteworthy differential expression of volatile metabolites was observed. The chromatographic profiling study afforded a total of 88 different volatile compounds. Healthy uninjured mango fruits yielded 10 metabolites as a standard and uninoculated injured mango fruit emitted 23 compounds. Mango fruits inoculated with *Colletotrichum gloeosporioides* yielded 29 volatile metabolites; while that inoculated with *Lasiodiplodia theobromae* Pat. yielded 26 different volatile metabolites. Among them only 3 volatile compounds (2,6,10-trimethyltetradecane, crocetane and phellandrene) found consistently in fruits inoculated with *C. gloeosporioides* and *L. theobromae*. Whereas, 2-propylmalonic acid, 1,4 cyclohexadiene, 1-methyl-, boronic acid, cyclohexyl dimethoxymethyl, Acetic acid, methyl ester, and Thujol was common in fruits inoculated with *C. gloeosporioides* while fruits inoculated with *L. theobromae* had specific volatiles 1,3-dimethoxy-2-(methoxymethyl)-2-methylpropane, Oxalic acid, cyclohexyldecyl ester, Cyclopentanepropionic acid, 2,5-cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-, Carbonic acid, octadecyl phenyl ester, Spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclopentane), 1',4',2,6,6-pentamethyl- and stearic acid. This study suggests that these unique metabolites can be used as biomarkers to detect postharvest diseases or toxigenic fungal pathogens of mango at an early stage of disease progression and to successfully device strategies to protect the fruits.

**Key words:** Discrimination, GC-MS, post harvest pathogens, profiling, volatilomics

## 1. Introduction

Mango is one of the world's most important and reputable fruits and is considered as the "King of fruits" or "Apple of tropics", probably originated from Indo Burma region (Mukherjee, 1971). India is a major producer and consumer of mango, followed by China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh and Nigeria (Sekhar *et al*., 2013). Mangoes are often stored for a few months after harvest in controlled environment which are conducive for post harvest pathogens (Kushalappa *et al*., 2007). Detection and discrimination of pathogens in stored mangoes is very difficult, especially in controlled atmosphere storage, where mangoes are kept in closed chambers for long duration of time.

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Around 27 different genera of fungi are known to cause diseases in mango fruits (Prakash and Srivastava, 1987) of which anthracnose caused by *C. gloeosporioides* Penz. (Penz. and Sacc) and stem end rot caused by *L. theobromae* Pat. (Griffon and Maubl.) are the most important (Johnson and Coates, 1993). One of the characteristic features of these post harvest diseases is that at some stage between the arrival of the inoculum and the development of progressive disease, growth of the pathogen is arrested. Such arrested infections are usually described as latent (Verhoeff, 1974). Anthracnose and stem end rot develop during ripening from latent infections that have occurred in the field. The detection of these diseases in storage is often possible only at an early stage and any treatment at this period will not significantly reduce losses. A controlled atmosphere storage method which consists of combining low temperature and oxygen with levels of carbon dioxide in order to suppress the metabolic activities of fruits in storage is one of the most important advances since beginning of the use of mechanical cooling methods in fruit and vegetable storage (Blanpied, 1987). But, under these conditions, the detection of diseases by visual observations is not possible since the storage room is closed for

longer periods of time, in addition to mangoes are buried in the pallet boxes. Thus, there is a need to develop sensitive, rapid and cost effective methods for the detection and identification of pathogens. Such a knowledge base can ultimately help reduction of unexpected storage losses.

Volatiles produced by rotting fruits have been identified and used by many researchers in order to develop a most sensitive technology to detect and distinguish diseases in stored perishable products. The production of volatile metabolites from diseased carrot, citrus, onion, peach, potato, raspberry and other crops have been reported by various workers using gas chromatography-mass spectrometry (GC-MS) (Kallio and Salorinne, 1990; Ouellette *et al*., 1990). The inoculation of mango with *C. gloeosporioides* and *L. theobromae* produced disease specific compounds (Kushalappa *et al*., 2007). Many compounds produced by pathogens have been identified. Though volatiles have been detected in diseased fruits and vegetables, the occurrence of these compounds seems to be quite discrepant. In spite of such fluctuations, modeling impendent has been taken to discriminate diseases. In mango, using neural network modeling of GC retention time and peak area data, it was possible to discriminate four diseases (Kushalappa *et al*., 2007). With this background, the present study was undertaken to analyze volatiles of mango cultivar Neelum, inoculated with a two important toxigenic fungal pathogens using GC-MS with a supreme aim to develop a disease warning system for use in controlled storage.

## 2. Materials and Methods

### 2.1 Pathogen source

The following fungal strains were used: *C. gloeosporioides* (ARAF) and *L. theobromae* (KKNT). The strains were collected from the infected mango fruits collected from Tamil Nadu State, India. The pathogens were identified both morphologically and by molecular approaches and submitted the sequenced data in NCBI database (KM269466 and KM508492). Also, pathogenicity of *C. gloeosporioides* and *L. theobromae* was proved by Koch's postulates on healthy mango fruit cv. Neelum (Parthasarathy *et al*., 2016).

### 2.2 Preparation of spore suspensions

The pathogens were purified and subcultured on the PDA. Then the cultures were supplemented with vitamin B and incubated at 24°C for 7 days. After attaining the full radial mycelial growth of pathogens on plates immediately exposed to near UV light for sporulation. The suspensions of conidia of both the pathogens were prepared from 7 to 12 days old cultures, by inundating the plates with sterile water, vortexing the suspension and filtering through cheese cloth. The suspension of *C. gloeosporioides* was manually adjusted to $10^5$ spores $ml^{-1}$ and similarly, *L. theobromae* was adjusted to $10^4$ spores $ml^{-1}$ using a haemocytometer under microscopic image analyzer (PCL Lab Instruments).

### 2.3 Inoculation and incubation

Healthy, even size and mature green mangoes of cv. Neelum were obtained from a mango growing area of Tamil Nadu state, India. Surface sterilized in 1 per cent sodium hypochlorite solution for 15 min and rinsed with sterile water thrice. Six holes were made in the equatorial region with a cork borer (3 mm diameter, 3 mm depth). Each hole was inoculated with 30 µl of *C. gloeosporioides* and 30 µl of *L. theobromae* spore suspension using a micropipette separately. Control fruits were spotted with sterile distilled water. Inoculated and control mangoes were placed separately in 2 litre capacity volatile trapping container connected with absorbing solvent, one fruit per container, with 15 ml of sterile water at the bottom to create humidity saturated atmosphere and incubated at 24°C in the dark. Stainless steel supports were used to suspend mangoes above water surface. After 24 h, the water was removed. Each experiment was repeated thrice.

### 2.4 Disease progression and observation

Lesion areas were measured and microscopic observations were made at 28, 52, 76 and 100 h. after inoculation (HAI). Three fruits per treatment, each with six inoculation spots were used per sampling period. The samples were subsequently observed by light microscope. The diameter and the depth of the diseased tissues were measured 6 days after inoculation (DAI) for *C. gloeosporioides* and 4 DAI for *L. theobromae*, from which the volume of diseased tissue was calculated.

### 2.5 Collection of head space volatile compounds

The head space volatile trapping unit is pictured in (Figure 2). There were four experimental fruits group, *i.e., C. gloeosporioides* infected fruits, *L. theobromae* infected fruits, uninoculated injured fruits and uninoculated healthy fruits. Within each group, there were three replications. The head space volatile trapping unit was placed under light intensity of 300 µmol $m^{-2}$ $s^{-1}$, at temperatures of 18°C at night and 26°C during the day, with a 14 litre: 10 day photoperiod. Both un-inoculated and inoculated fruits were incubated at 100 per cent R.H. for 26 h under a constant airflow of 25 ml $min^{-1}$ (Laothawornkitkul *et al*., 2008). Head space volatile sampling was performed during the light period at daily intervals. There were several trapping periods, *i.e*., 1-7 days for *C. gloeosporioides*, 1-5 days for *L. theobromae* and 1-7 days for uninoculated injured and healthy fruits. In between each trapping period, humid air was supplied through each vessel at a constant airflow of 25 ml $min^{-1}$ per vessel. During day time, the relative humidity was about 40-60 per cent and during the night time, it was constant at 100 per cent. Before each volatile trapping period, the humidifier was replaced by trapping filters consisting of 250 ml silica gel, 250 ml molecular sieve 4 Å, and 1000 ml activated charcoal. All of the treatments were purged and trapped in parallel with airflow of 60 ml $min^{-1}$ per container. The non reactive bottle containing *n*-Hexane (HPLC grade) was connected into the container to sample headspace gas. The accumulated headspace gas was trapped by *n*-Hexane (HPLC grade) solvent, concentrated and diluted in three different dilutions and analysed using GC-MS (Pritiviraj *et al*., 2004). The experiments were repeated twice.

### 2.6 Gas chromatography-mass spectrometer (GC-MS) analysis

The 24 samples from four trapping periods were analyzed by coupled gas chromatography-mass spectrometry (GC-MS) in Department of Nano Science and Technology, Tamil Nadu

Agricultural University, Coimbatore. Two µl of each sample was injected into the GC-MS system. This system was based on a gas chromatograph (Thermo scientific Trace GC Ultra DSQ II) equipped with a 30 m " 0.25 mm internal diameter, HP5-MS column with 0.25 mm film thickness. under the following conditions: carrier gas as Helium with flow rate of 1 ml per minute and 1 µl sample injection with pre injection of solvent by AI/AS 3000 Method; split-less mode injection with 30 sec of sampling time; initially the column temperature was maintained at 50°C at the increasing rate of 10°C/min no hold was followed by increasing up to 200°C and kept at the same temperature for 2 min hold with 3kPa surge pressure and 220 base temperature at right split/split less injector method and 250 base temperature at right electron capture detectors method with the AUX 1 pressure controlled MS transfer line at 250°C; the electron impact energy was 70eV, Julet line temperature at 2000°C and the source temperature at 200°C. Electron impact (EI) mass scan (m/z) was recorded in the 45-450 aMU range.

### 2.7 Identification and quantification of volatile metabolites

The identification chromatographic peak was carried out by comparing their mass spectra with those of the bibliography of unknown compounds from the NIST 14 MS data library through online mass spectra database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000). The approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software. The results are presented as the peak area normalized per cent and discussed in detail with cited references.

## 3. Results

### 3.1 Pathogen

Isolated necrotrophic fungal strain of *C. gloeosporioides* produced grayish white color raised fluffy mycelial growth with smooth margin on potato dextrose agar (PDA) plates. Conidium was unicellular, straight, cylindrical, and obtuse at the apex, sometimes acute at the base and 10.00 - 22.50 3.75 - 6.25 µm in size (Figure 1a). In regards to *L. theobromae* aerial blackish grey colony with concentric zonation on PDA plates, immature conidia were hyaline, ellipsoidal to subovoid, amerospore, 22.73 - 27.04 × 11.88 -15.84 µm, thick-walled with a granular content. Mature conidia were dark brown, ellipsoid to ovoid, didymospore, 19.66 - 26.35 × 11.30 - 14.17 µm, with longitudinal and irregular striations (Figure 1b).

### 3.2 Disease progress

The diameters of lesions of *L. theobromae* inoculated mangoes were 3.0 and 5.0 cm (including cork borer wound) at 3 and 4 DAI, respectively. Among *C. gloeosporioides* inoculated mangoes, the lesion diameter was 0.8, 1.9 and 3.2 cm at 3, 4 and 6 DAI. The volume of the stem-end rot diseased tissue at 4 DAI was 21.5 $cm^3$ and for anthracnose at 6 DAI was 9.1 $cm^3$. So, the progress of *L. theobromae* was faster and deeper on mango cv. Neelum (volume = 21.5 $cm^3$) compared to *C. gloeosporioides* (volume = 9.1 $cm^3$).

### 3.3 Volatile metabolites

Toxigenic fungal pathogens of spoilt mango fruits were trapped and profiled. The fungal pathogens include *C. gloeosporioides* (ARAF) and *L. theobromae* (KKNT). They were inoculated into unripe healthy mango fruits. Volatile metabolite profile of healthy ripe mango fruits was determined by GC-MS analysis of its *n*-Hexane solvent trapping method and the result presented (Table 1). From the result, ten metabolites were determined. Predominated among them were: 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (46.19%), β-pinene (5.04%) Myrcene (3.34%), and the least was 5-isopropyl-3,8-dimethyl-1,2,4,5,6,7-hexahydroazulene (0.37%) (Figure 3). The results of GC-MS analysis of the *n*-Hexane extract of mango uninoculated injured fruits were presented (Table 2). From the result, twenty three metabolites were determined predominated by 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (55.61%), followed by Tritetracontane (4.95%), 1-Octacosanol (3.07%) and n-Tetradecane was the least (0.33%) (Figure 4). GC-MS analysis of the *n*-Hexane extract of mango fruits inoculated with *C. gloeosporioides* was conducted and the results presented (Table 3). From the result, twenty nine metabolites were determined predominated by Mono (2-ethylhexyl) phthalate (21.05%), followed by myristic acid (5.86%), n-Cetane (3.77%), 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (3.43%), thujol (3.02%) and phthalic acid, ethyl pentadecyl ester was the least (0.28%) (Figure 5). GC-MS analysis of the *n*-Hexane extract of mango fruits inoculated with *L. theobromae* was conducted and the result presented (Table 4). From the results, twenty six metabolites were determined predominated by 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (49.59%), followed by 1,3-Dimethoxy-2-(methoxymethyl)-2-methylpropane (8.65%), Oxalic acid, cyclohexyldecyl ester (3.38%) and Stearic acid was the least (0.23%) (Figure 6).

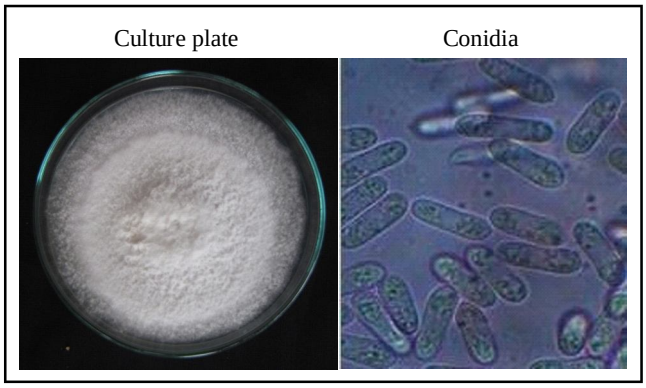


**Figure 1a:** Morphological characters of *C. gloeosporioides*.

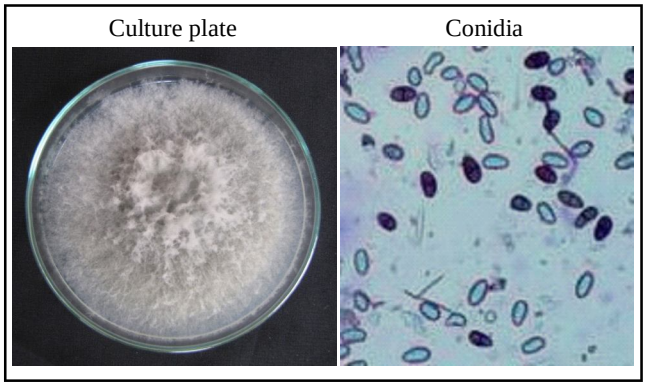


**Figure 1b:** Morphological characters of *L. theobromae*.

**Table 1:** Result of GC/MS analysis of uninoculated healthy mango fruit

| $RT^{-1}$ (min) | Volatile metabolites | Peak area normalized (%) |
|---|---|---|
| 8.34 | n-Undecane | 3.29 |
| 11.17 | β-pinene | 5.04 |
| 14.91 | 2,6,11-Trimethyldodecane | 3.29 |
| 18.16 | Myrcene | 3.34 |
| 21.24 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester | 46.19 |
| 21.53 | 2-carene | 1.59 |
| 21.91 | 2,6,11-Trimethyldodecane | 1.33 |
| 23.34 | 5-isopropyl-3,8-dimethyl-1,2,4,5,6,7-hexahydroazulene | 0.37 |
| 25.06 | caryophyllene | 0.79 |
| 32.78 | Myristic acid | 1.85 |

**Table 2:** Result of GC/MS analysis of uninoculated injured mango fruit

| $RT^{-1}$ (min) | Volatile metabolites | Peak area normalized (%) |
|---|---|---|
| 3.45 | 4,4-Dimethyl-2-pentene | 0.55 |
| 5.77 | n-Undecane | 0.63 |
| 7.16 | n-Decane | 0.46 |
| 9.13 | Silane, cyclohexyldimethoxymethyl- | 0.62 |
| 13.09 | n-Tetradecane | 0.33 |
| 13.72 | 2,7,10-Trimethyldodecane | 0.63 |
| 15.55 | 2,6,11-Trimethyldodecane | 0.44 |
| 19.28 | 4-Methyltetradecane | 0.6 |
| 20.89 | 2,6,10,14-Tetramethylheptadecane | 0.89 |
| 22.11 | 2,6,11-Trimethyldodecane | 1.46 |
| 23.78 | Crocetane | 0.76 |
| 29.55 | Caryophyllene | 1.15 |
| 31.34 | 2,6,10-Trimethyltetradecane | 1.33 |
| 32.03 | Myristic acid | 1.36 |
| 34.38 | 1-(4-Bromobutyl)-2-piperidinone | 0.7 |
| 36.78 | 2,6,10-Trimethyltetradecane | 1.04 |
| 43.52 | 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl)ester | 55.61 |
| 45.05 | 1,1-Dimethyltetradecyl hydrosulfide | 0.85 |
| 47.86 | 1-Octacosanol | 3.07 |
| 48.31 | Tritetracontane | 4.95 |
| 49.25 | 9-Octadecenamide, (Z)- | 2.27 |
| 51.90 | Tetrapentacontane, 1,54-dibromo- | 0.42 |
| 52.51 | 1-Hexacosene | 2.26 |

**Table 3:** Result of GC/MS analysis of *C. gloeosporioides* infected mango fruit

| $RT^{-1}$ (min) | Volatile metabolites | Peak area normalized (%) |
|---|---|---|
| 4.73 | 2-Propylmalonic acid | 0.94 |
| 5.73 | 1,4 Cyclohexadiene, 1-methyl- | 1.72 |
| 7.14 | 4,5-Dimethylnonane | 1.39 |
| 9.11 | Cyclohexyldimethoxymethyl | 1.46 |
| 9.27 | Acetic acid, methyl ester | 1.63 |
| 13.09 | 3,7-Dimethyldecane | 0.82 |
| 13.72 | 2,7,10-Trimethyldodecane | 1.49 |
| 15.53 | 2,6,11-Trimethyldodecane | 1.15 |
| 18.44 | 2-Butanoic acid, methyl ester | 0.99 |
| 19.28 | 4-Methyltetradecane | 0.56 |
| 21.87 | n-Cetane | 3.77 |
| 22.11 | 1,4-pentadiene | 1.06 |
| 23.80 | Crocetane | 1.02 |
| 26.00 | Hexadecanoic acid, ethyl ester | 0.65 |
| 28.12 | 2,6,10-Trimethyltetradecane | 0.83 |
| 28.57 | 2,6,10-Trimethyltetradecane | 1 |
| 29.57 | Boronic acid, ethyl- | 2.39 |
| 32.38 | Myristic acid | 5.86 |
| 33.12 | Phellandrene | 1.14 |
| 36.17 | 3-carene | 0.66 |
| 36.80 | 2,6,10,15-Tetramethylheptadecane | 1.18 |
| 38.41 | Thujol | 3.02 |
| 40.94 | 2,6,10-Trimethyltetradecane | 0.49 |
| 41.73 | Phthalic acid, ethyl pentadecyl ester | 0.28 |
| 42.24 | 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester | 3.43 |
| 42.89 | Mono(2-ethylhexyl) phthalate | 21.5 |
| 47.19 | 2-Octadecoxyethanol | 1.14 |
| 47.80 | n-Eicosane | 1.91 |
| 50.43 | 2,6,10-Trimethyltetradecane | 0.98 |

**Table 4:** Result of GC/MS analysis of *L. theobromae* infected mango fruit

| $RT^{-1}$ (min) | Volatile metabolites | Peak area normalized (%) |
|---|---|---|
| 2.41 | 1,3-Dimethoxy-2-(methoxymethyl) -2-methylpropane | 8.65 |
| 3.47 | Oxalic acid, cyclohexyldecyl ester | 3.38 |

| | | |
|---|---|---|
| 4.06 | Cyclopentanepropionic acid | 0.66 |
| 5.75 | n-Undecane | 1.37 |
| 7.14 | 4,5-Dimethylnonane | 0.66 |
| 9.11 | Durene | 1.01 |
| 13.09 | n-Tetradecane | 0.73 |
| 13.72 | Silanol, trimethyl- | 1.25 |
| 15.53 | 2,6,11-Trimethyldodecane | 0.42 |
| 19.28 | n-Pentadecane | 0.59 |
| 20.69 | 2,5-Cyclohexadiene-1,4-dione, 2,6 -bis(1,1-dimethylethyl)- | 2.2 |
| 22.11 | 2,6,11-Trimethyldodecane | 2.59 |
| 23.78 | Carbonic acid, octadecyl phenyl ester | 0.79 |
| 27.18 | Ethyl boronate | 0.45 |
| 29.53 | 5-(4-Trifluormethylsulfonylphenylazo) -1,2,3,4,5-pentamethylcyclopentadiene | 0.48 |
| 29.81 | Crocetane | 1.97 |
| 31.30 | Spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclo [2.1.0]pentane), 1',4',2,6,6-pentamethyl- | 0.79 |
| 34.40 | 2,6,10-Trimethylpentadecane | 0.64 |
| 36.48 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6, 9-diene-2,8-dione | 0.62 |
| 36.78 | 2-Methylnonadecane | 1.39 |
| 38.15 | Hexadecanoic acid | 0.93 |
| 42.28 | Phellandrene | 1.55 |
| 43.40 | 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester | 49.59 |
| 44.18 | Stearic acid | 0.23 |
| 48.01 | 2,6,10-Trimethyltetradecane | 1.27 |
| 50.45 | Phthalic acid, butyl tetradecyl ester | 0.51 |

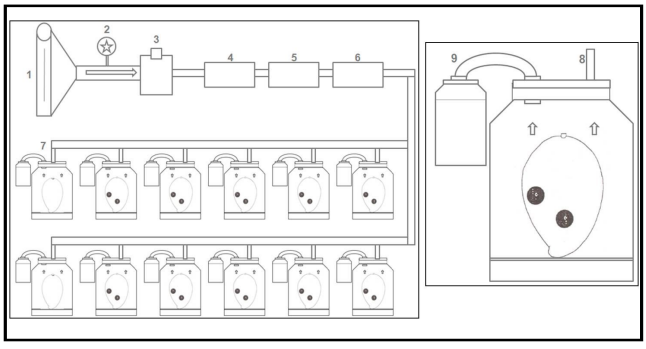


**Figure 2:** Diagram of the volatile trapping unit: (1) air blower, (2) flow meter, (3) humidifier, (4) silica gel, (5) molecular sieve, (6) activated charcoal, (7) container contain fruit, (8) air inlet and (9) volatile trapping container contain *n*-Hexane (HPLC Grade).

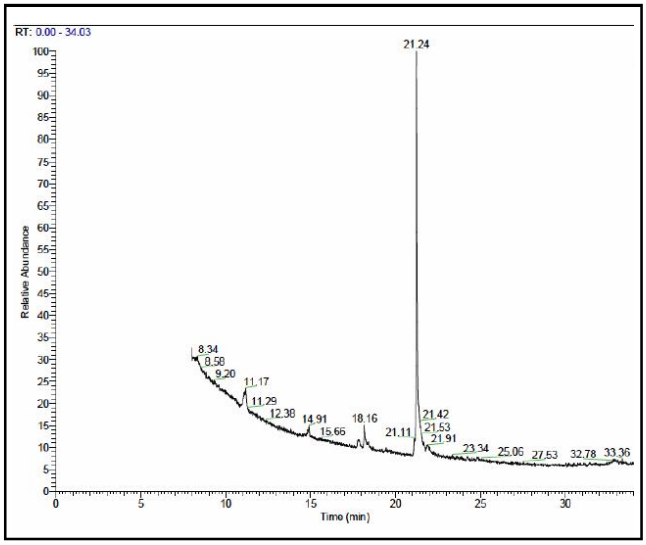


**Figure 3:** GC-MS analysis of uninoculated healthy mango fruit.

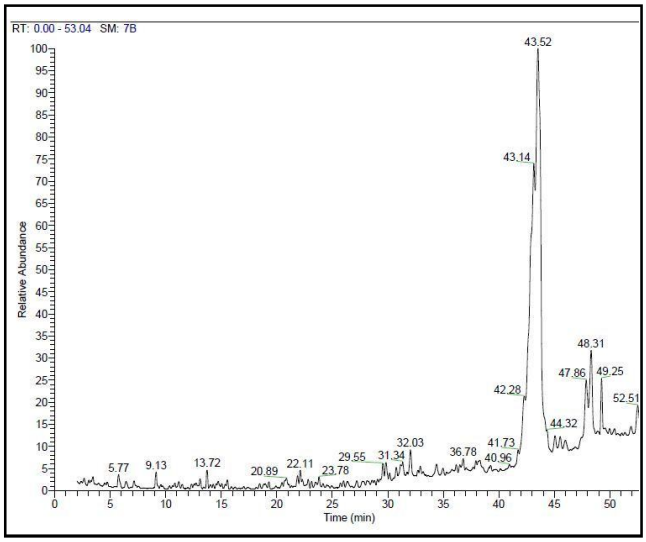


**Figure 4:** GC-MS analysis of uninoculated injured mango fruit.

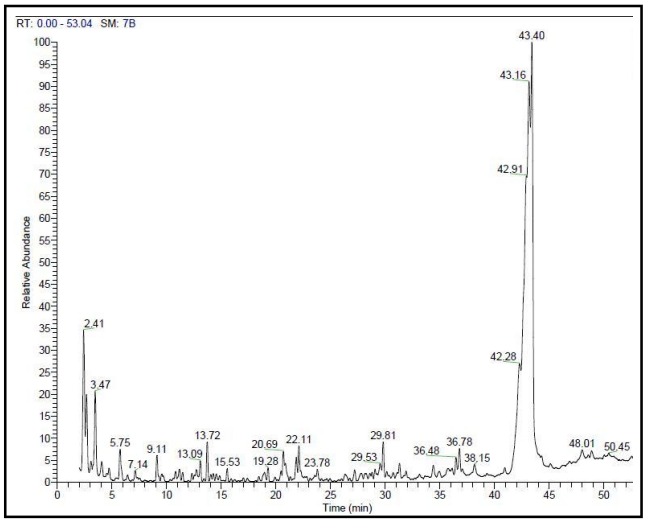


**Figure 5:** GC-MS analysis of C. gloeosporioides infected mango fruit**.**

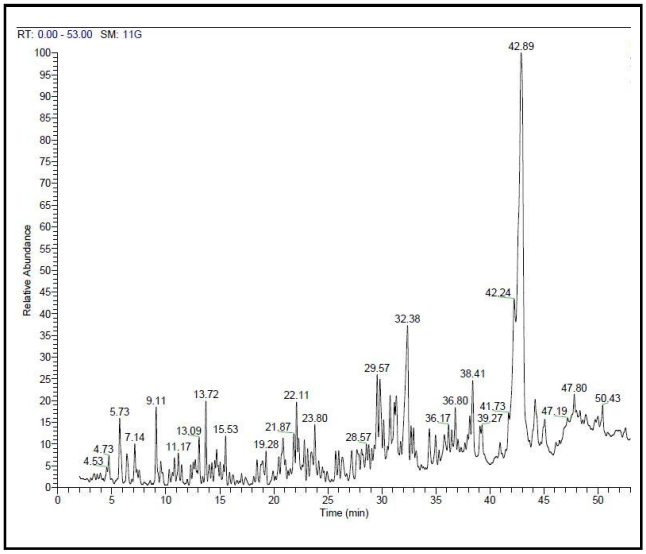


**Figure 6:** GC-MS analysis of *L. theobromae* infected mango fruit.

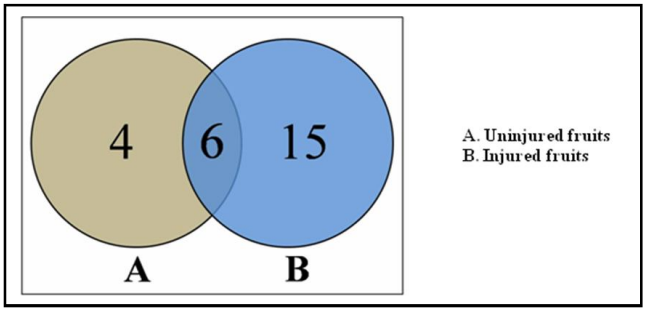


**Figure 7:** Volatile produced by uninjured and injured fruits.

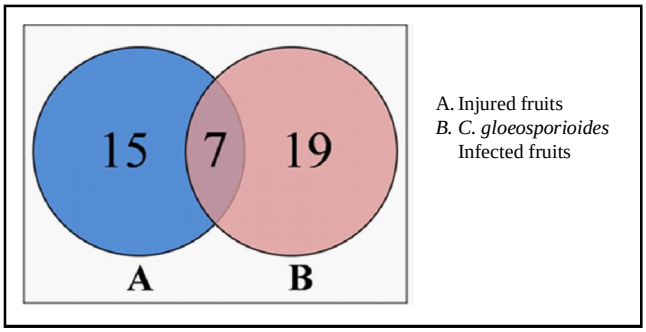


**Figure 8:** Volatile produced by injured and *C. gloeosporioides* infected fruits.

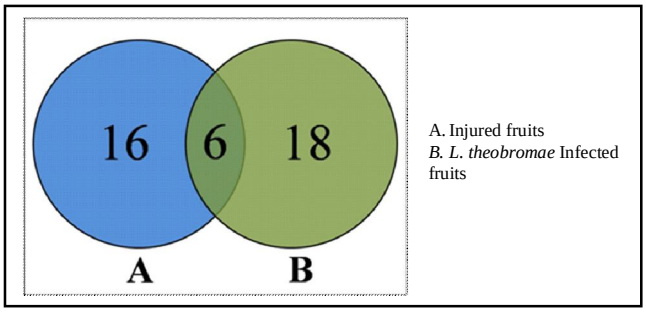


**Figure 9:** Volatile produced by injured and *L. theobromae* infected fruits.

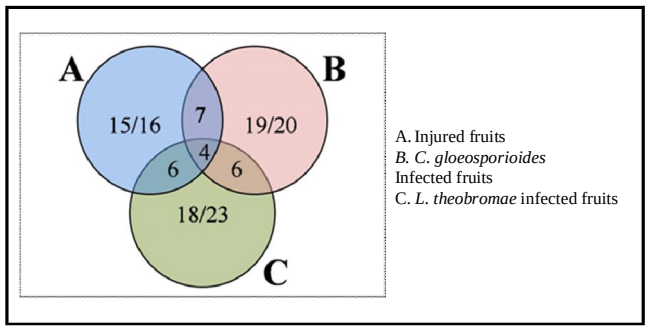


**Figure 10:** Volatiles produced by injured, *C. gloeosporioides* and *L. theobromae* infected fruits.

## 4. Discussion

This is the first novel study to provide data on the composition of the *n*-Hexane extract volatile metabolites of mangoes inoculated with pathogens and provides the basis for discriminating the major post harvest diseases. Several compounds were unique to a disease/ inoculation, which could be qualitatively used to discriminate diseases studied here, in known disease samples. 2-Propylmalonic acid; Acetic acid, methyl ester; Boronic acid, ethyl- and Thujol were unique to *C. gloeosporioides* inoculated mangoes, which could be discriminated from *L. theobromae* ones, which also produced unique metabolites, 1,3-Dimethoxy-2-(methoxymethyl)-2-methylpropane; Oxalic acid, cyclohexyldecyl ester; Durene; 2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-; Carbonic acid, octadecyl phenyl ester; 5-(4-Trifluormethyl sulfony lphenylazo)-1,2,3,4,5-pentamethylcy clopentadiene; Spiro-1-(cyclohex-2-ene)-2'-(5'-oxabicyclo[2.1.0] pentane), 1',4',2,6,6-pentamethyl-; 7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione and Stearic acid.

These unique metabolites can be used as biomarkers to detect the presence of these toxigenic fungi in spoilt mango fruits. Disease-specific metabolites have been detected in many diseased fruits and vegetables in stored conditions. In a study on apples, methyl acetate was detected to be unique to fruits inoculated with *Botrytis cinerea*, 4-methyl-1-hexene to fruits inoculated with pathogenic, *Mucor piriformis*, 2-methyltetrazole and butyl butanoate to fruits inoculated with blue mould fungi, *Penicillium expansum*, and 3,4-dimethyl-1-hexene and fluorethene to fruits inoculated with *Monilinia* sp. (Vikram *et al*., 2004a). 1- Pentanol and ethyl boronate were also reported to be unique for bacterial soft rot of carrot (Vikram *et al*., 2006). Similarly, in one (1)-pentanol and ethyl boronate, were detected in *L. theobromae* inoculated mangoes alone, while thujol was observed only in *C. gloeosporioides* inoculated mangoes (Moalemiyan *et al*., 2006). Acetyl hydrazide, propylcarbamate, propenyl bromide, acetone, 1-ethenyl-4-ethyl benzene, thiirane and 1-(methylthio)-E-1-propene were unique to onion bulbs artificially inoculated with *Botrytis allii*, while 3-bromo furan was specific to bulbs inoculated with *E. carotovora* subsp. *carotovora* (Prithiviraj *et al*., 2004). Also, seven unique compounds, *viz*., 1-pentanol, 3-methylbutanol, 2-methylpropanol, 2, 3-butanedione, ethyl boronate, isopentyl methyl ether and ethane

ethoxy were detected in carrots (cv. Vita Treat) inoculated with *E. carotovora* subsp. *carotovora* (Vikram *et al.*, 2006). The use of unique compounds for disease discrimination may be valid if, the lesions are spatially separated, but their uses when the diseases occur together in the same lesion remain to be validated.

Comparatively, some similar par volatile metabolites synthesized among uninoculated healthy and uninoculated injured fruits. Especially n-undecane, 2,6,11-Trimethyldodecane, Caryophyllane, 2,6,10-Trimethyltetradecane and 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (Figure 7). Similarly, par data of volatile metabolites produced among injured versus *C. gloeosporioides* infected mangoes and injured versus *L. theobromae* infected mangoes were presented in Figure 8 and Figure 9.

The metabolites 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester, 2,6,11-Trimethyldodecane, 2,6,10-Trimethyltetradecane, n-undecane and crocetane were common to all the experimental mangoes, but n-undecane were absent in the *C. gloeosporioides* inoculated mangoes (Figure 10). Even though, these compounds were common to all the treatments, differences in their relative abundance can help to detect and discriminate diseases or presence of toxigenic fungi, especially in the absence of unique or other disease discriminatory volatile compounds. *L. theobromae* inoculated mangoes had the highest amount of Hexadecanoic acid (0.93 per cent) than *C. gloeosporioides* inoculated mangoes which had 0.65 per cent. The absence of these compounds in the healthy and injured mango fruits agreed (Yilmaz, 2001). The presence and / or absence of the above metabolites and the variations in their abundance could be considered for qualitative discrimination of fungal pathogens, *viz*., *C. gloeosporioides* and *L. theobromae*, especially when unique compounds are absent and mixed infections, especially in the same lesion, are present.

The volatile metabolites produced by mangoes belong to several chemical groups: monoterpenes, sesquiterpenes, esters, aldehydes, ketones, alcohols, organic acids, aliphatic hydrocarbons and aromatics (Shibamato and Tang, 1990). The majority of total aroma volatile compounds from mango are hydrocarbons, monoterpenes or sesquiterpenes (Winterhalter, 1991). The mutual exclusiveness of exogenous metabolites among replicates has been reported in previous studies on some other crops (Prithiviraj *et al*., 2004; Vikram *et al*., 2004; Lui *et al*., 2005; Vikram *et al*., 2006). Such variation is also not unusual in endogenous metabolic profiling studies (Roessner *et al*., 2001). Although, there are several reports of production of volatiles in healthy mangoes, no work has yet been published on the production of volatile metabolites by diseased mangoes. Misidentification of secondary metabolites using the NIST library, especially using mass ions in the minimal limited range of 46-300 *m/z*, maturity stage of mangoes, extraction solvent and method could assign to the variation in number and occurrence of secondary metabolites. Reactions among the different volatiles and also between volatiles of fruits or vegetables have been reported as other potential reasons for variability in volatile profiles among replicates (Hamilton-Kemp *et al*., 1996).

## 5. Conclusion

Volatile analysis will continue to be applied for the identification of hazardous pathogens in perishable commodities. Many pathogens are important in storage environment; the outbreaks are often from a single disease. The toxigenic fungi/disease-specific volatile metabolites, unique and common to only *C. gloeosporioides* and *L. theobromae* reported here, could be used as biomarkers to discriminate diseases / toxigenic fungi even when more than one disease is present, but this has to be tested before commercial. This might lead to a novel way of plant pathogen detection.

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### Conflict of interest

We declare that we have no conflict of interest.

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