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Profiling of Secondary Metabolites Produced by *Colletotrichum coccodes* Pathogen of Tomato (*Solanum lycopersicum* L.) via Gas Chromatography–Mass Spectrometry (GC-MS)

Puma S.H[1*], Chimbekujwo I.B.[2], Zakari B.G.[3], Abdullahi M.[4] & Bristone B.[5]
[1]Department of Horticultural Technology, Federal College of Horticulture Dadin-kowa Gombe State
[2,3&5]Department of Plant Sciences, Modibbo Adama University Yola, Adamawa State, Nigeria
[3]Nigeria Natural Medicine Development Agency, Lagos
[*]Corresponding Author Email: serahpuma@gmail.com

ABSTRACT
Studies on mycotoxins produce by *Colletotrichum coccodes* a pathogenic fungus responsible for anthracnose disease of tomato (*Solanum lycopersicum*) was conducted. *C. coccodes* was consistently isolated from three varieties of tomato on potato dextrose agar. For mycotoxin extraction the isolated and identified fungi was cultivated on Potato dextrose broth for 21 days and was sequentially extracted using methanol and n-hexane. Then the excess solvent was evaporated from the extract in a fume hood. The dried extract was then channeled to gas chromatography–mass spectrometry (GC-MS) analysis. The GC-MS chromatogram revealed the following secondary metabolites based on the corresponding peaks, which includes: n-hexadecanoic acid $C_{16}H_{32}O_2$, RT 13.55 min, 19.0% area), methyl palmitate $C_{17}H_{34}O_2$, RT 13.18 min, 9.7%), cis-9-octadecenoic acid, $C_{18}H_{34}O_2$, RT 13.74 min, 3.1%), methyl oleate ($C_{19}H_{36}O_2$, RT 14.59 min, 7.8%), 9,12-octadecadienoic acid, $C_{18}H_{32}O_2$, RT 14.55 min, 2.4%), and the p-Menth-8(10)-en-9-ol, $C_{10}H_{18}O$, RT 14.38 min, 1.3%). Many identified compounds have been reported to possess antimicrobial or insecticidal activities. These results provide the first GC-MS metabolite fingerprint for *C. coccodes* in this part of the world. The biological relevance of these compounds (innate antimicrobial effects) is discussed.

Keywords:
GC-MS,
Secondary Metabolite,
Colletotrichum
Coccodes,
Tomato

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are a very important crop globally, holding the second-largest cultivation area after potatoes and leading all vegetables in processing use. The past five decades has seen a substantial increase in tomato farming, a trend that aligns with the current global emphasis on sustainable organic food production (Pinho *et al*., 2011). This growth is partly attributed to their crucial role as a major supplier of essential vitamins and minerals, contributing to a notable 10% rise in yields (Bertin & Génard, 2018).

Tomatoes are a recognized nutritional powerhouse, providing essential nutrients vital for overall health and wellness (Martínez et al., 2024). They are particularly rich in vitamin C, folate, potassium, and vitamin K, contributing significantly to a balanced diet. A defining characteristic of tomatoes is their high lycopene content, a powerful antioxidant responsible for their vibrant red color and its health benefits that may help protect against diseases such as cancer and cardiovascular conditions (Coelho *et al*., 2023).

Nutritionally, tomatoes are remarkably low in calories: a 100-gram serving contains only 18 calories, primarily composed of 95% water. This serving also provides 0.9 grams of protein, 3.9 grams of carbohydrates (including 2.6 grams of sugar), 1.2 grams of fiber, and a minimal 0.2 grams of fat (Wang *et al*., 2023). This low-calorie, high-water composition makes them not only palatable in diverse culinary uses but also a hydrating and beneficial dietary choice, ultimately contributing to individual health and vitality (Coelho *et al*., 2023).

Despite advancements in tomato production, the crop remains susceptible to fungal diseases, which are a major contributor to plant ailments and substantial yield reductions (Kiralan & Ketenoglu *et al*., 2022). Common fungal diseases affecting tomatoes include leaf blights, leaf spots, mildews, rots, and wilts, caused by fungal species such as *Alternaria solani*, *Septoria*, *Phytophthora infestans*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Fusarium oxysporum* and Colletotrichum species. *Colletotrichum cooccodes* is the most prevalent species affecting tomato fruit,

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Others like *C. truncatum, C. gloeosporioides, C. acutatum, C. dematium, C. fioriniael, and C. nymphaeae*. Fungi are responsible for around 85% of plant diseases, and in tomatoes, this can translate to 50-80% fruit loss without effective management (Khan *et al*., 2023). The management of fungal diseases in tomatoes is crucial to prevent losses and ensure sustainable production. According to Rothan *et al.* (2021), some species of fungus produce mycotoxins that are very toxic to humans.

Mycotoxins, a varied group of secondary metabolites produced by filamentous fungi such as *Aspergillus*, *Penicillium*, *Alternaria*, and *Fusarium*, are a major public health concern due to their association with a range of adverse health effects (Shekar *et al*., 2025). They have even been classified as the most important chronic dietary risk factor, above synthetic contaminants, plant toxins, and pesticide residues (Zinedine *et al*., 2007). The diverse nature of mycotoxins results in a wide array of acute and chronic toxic effects in humans and animals, encompassing symptoms like skin irritation, feeding refusal, nausea, vomiting, diarrhea, anemia, hemorrhage, and immunosuppression (Marin *et al*., 2013). The present study is focused on the identification of mycotoxins produced by *Colletotrichum coccodes* using Gas chromatography and mass spectrometry (GC-MS).

MATERIALS AND METHODS

Study Area

The research work was conducted in two study areas; Adamawa State latitude 9.2092º N and longitude 12.4823º E and Gombe State latitude 10.2983º E and longitude 11.1773º E, the two areas lie under the Northern Guinea Savannah ecological zone of Nigeria between the latitude 8º 47 to 9º 19'N and longitude 11º 09 to 12º 30 E. Both locations have tropical continental type climate characterized by well-marked wet and dry seasons. The wet seasons usually begins around late April and ends in October, while the dry seasons begins in November and ends in March (Adebayo, 2021). Both locations have mean rainfalls of about 1,200mm and annual mean temperature of about 29º C with relative humidity ranges between 60-70 percent during the wet season to about 35-45 percent in the dry seasons (MapSof.net), the soil predominantly sandy and loamy in nature.

Fungal Culture and Mycotoxin Extraction.

Samples of tomato leaves, stems, and roots were collected from tomato farms in Gombe and Adamawa states. Samples from Gombe State (GM) with the following voucher numbers: Leaf: TSA23-C1-001-GM-L, Stem: TSA23-C1-001-GM-S and Root: TSA23-C1-001-GM-R and Samples from Adamawa State (AD) with the following voucher numbers: Leaf: TSA23-C1-002-AD-L, Stem: TSA23-C1-002-AD-S and Root: TSA23-C1-002-AD-R were isolated on Potato dextrose Agar (PDA). After 72 hours of incubation, the samples were sub cultured to obtained a pure culture. A piece mycelium was then placed on a microscope slide followed by the addition of two drops of lactophenol cotton blue and observed under the microscope at X40. *Colletotrichum coccodes* was identified as the pathogen responsible for the anthracnose disease of tomato in the study area. For the extraction of mycotoxins 0.5mm of *C. coccodes* mycelia was introduced into a 500 mL conical flask filled with 250 mL of potato dextrose broth using a cork borer and was incubated for 21 days at 25 °C. After incubation, the fungal culture and medium were homogenized and extracted with methanol and n-hexane by shaking on an orbital shaker at 200 rpm for 1 h at 25ºC. The organic extract was filtered, concentrated, and dried using liquid nitrogen.

GC-MS ANALYSIS.

Five hundred milligrams (500 mg) of the dried extract of *C. coccodes* were weighed and put into 50 mL conical flasks. Then, 10 mL of acetonitrile was added into the conical flask containing the extract was shaken on an orbital shaker for 24 hours to ensure complete dissolution. Finally, the extract was filtered using membrane filters (Sartorius NY 0.45 µm, 47 mm), and the filtrate was transferred into sterile and well-labelled 1.5 mL GC glass vials (Amber) before the GC-MS analyses.

The Gas chromatography (GC)/tandem mass spectrometry (MS) analysis of the fungal mycotoxins was performed on Agilent HP 7890 A with an Agilent 7000 triple quadrupole detector equipped with an Agilent 7683 auto-sampler using a capillary Zebron SemiVolatiles (30 m x 0.25 mm x ID 0.25 mm) column (Phenomenex®, CA, USA). Oven program: initial oven temperature 70 °C held for 1 min then at 40 °C/min ramped up to 120 °C, at 5 °C/min ramped up to 310 °C. The entire chromatographic run, including backflush, last for 46 min. The injector was set at 250 °C in the split-splitless mode (split vent flow 60 mL/min; split less time 0.75 min). Helium was used as carrier at a constant flow of 1.4 mL/min. The data acquisition and processing were performed using MassHunter® software from Agilent Technologies®. The MS scanned from m/z 50–600. Compound identification was achieved by comparing mass spectra and retention indices to the NIST 14 library. The relative area percentage of each peak (normalized to the total ion current) was used as an estimate of relative abundance (Bristone *et al*. 2022).

DATA ANALYSIS.

Identifications with library match quality >90% were accepted when supported by peak purity. The molecular formulae of compounds were confirmed by their CAS registry or chemical name (see Table 1). Known

biological activities of each compound were obtained from the literature and are cited below.

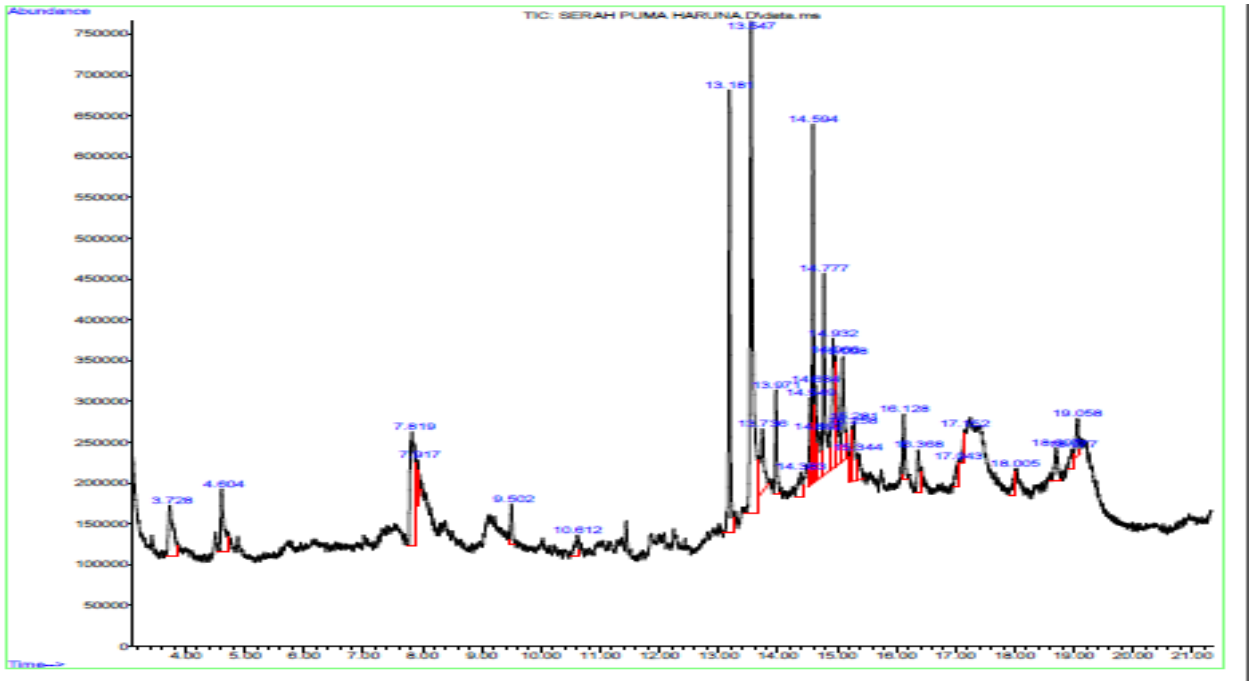

Figure 1: GCMS chromatogram of *Colletotrichum coccodes*

Table 1. Summary of selected compounds identified by GC-MS in *C. coccodes* extracts, Retention time, Area % with reported biological activities.

Name of Compound	Retention Time	Area (%)	Formula	Reported Biological Activity with their Reference
Butyl 2,5,8,11-tetraoxatridecan-13-oate	3.728	3.49	$C_{13}H_{26}O_6$	No specific bioactivity documented
Methyl benzoate (Benzoic acid, methyl ester	4.604	2.84	$C_8H_8O_2$	Insect attractant/pesticidal compound (Prasath *et al*., 2020).
4-Nitrobenzaldehyde 7	7.819	7.03	$C_7H_5NO_3$	Chemical intermediate used in synthesis (no reported antimicrobial activity)
Methyl 10-methylundecanoate (branched C_{12} FAME)	9.502	1.09	$C_{13}H_{26}O_2$	Saturated fatty acid ester – no notable antimicrobial activity
(Z,Z)-10,12-Hexadecadien-1-ol acetate	10.612	1.18	$C_{18}H_{32}O_2$	Insect semiochemical (pheromone); no known pharmacological activity found (Prasath *et al*., 2020).
Methyl 14-methylpentadecanoate (branched C_{16} FAME)	13.181	9.65	$C_{17}H_{34}O_2$	Branched fatty acid ester – limited data, no specific bioactivity reported
n-Hexadecanoic acid (Palmitic acid)	13.547	19.02	$C_{16}H_{32}O_2$	Antifungal (Candida) and anti-inflammatory fatty acid (Prasath *et al*., 2020).
Oleic acid (cis-9-octadecenoic acid)	13.736	3.11	$C_{18}H_{34}O_2$	Antifungal (Candida) and anti-inflammatory fatty acid (Ana *et al*., 2022).

Isopropyl palmitate	13.971	2.60	$C_{18}H_{34}O_2$	Common cosmetic emollient (no significant antimicrobial activity) (Shehu *et al*., 2025).
cis-p-Menth-8(10)-en-9-ol (Carveol)	14.383	1.25	$C_{10}H_{18}O$	Monoterpene alcohol; strong antimicrobial activity (active vs. E. coli, etc.) (Ainane *et al*., 2023).
9,12-Octadecadienoic acid (Linoleic acid)	14.549	2.37	$C_{18}H_{32}O_2$	Antibacterial (inhibits bacterial fatty-acid synthase) (Prasath *et al*., 2020).
trans-13-Octadecenoic acid, methyl ester (C18:1 FAME)	14.594	7.80	$C_{19}H_{36}O_2$	Unsaturated fatty ester; antibacterial activity via FabI inhibition (Prasath *et al*., 2020).
(Z,Z)-7,11-Hexadecadienal	14.692	1.61	$C_{16}H_{28}O$	Insect sex pheromone (e.g. citrus leaf miner) (Prasath *et al*., 2020).
Methyl stearate	14.777	6.23	$C_{19}H_{38}O_2$	Saturated fatty ester; minimal antibacterial (saturated C18) (Prasath *et al*., 2020)
(2E,13Z)-2-Methyl-3,13-octadecadien-1-ol	14.932	4.53	$C_{19}H_{36}O$	Terpenoid alcohol; reported larvicidal/acaricidal (mosquito larva, etc.) (Ana *et al*., 2022)
7-Pentadecyne	15.344	1.25	$C_{15}H_{28}$	No known bioactivity documented
Methyl 9-heptadecenoate (C18:1 FAME)	18.697	1.70	$C_{18}H_{34}O_2$	Unsaturated fatty ester; antibacterial activity via FabI inhibition (Liszkowska *et al*., 2023).
2,2-Dimethylpropanoate, 2,6-dimethylnon-1-en-3-yn-5-yl ester	18.977	0.99	$C_{16}H_{26}O_2$	Complex branched aliphatic ester; no known bioactivity documented
Oleic acid (cis-9-octadecenoic acid)	19.058	1.56	$C_{18}H_{34}O_2$	Essential fatty acid: anti-inflammatory properties, for dermatological applications in cosmetic formulations (Shehu *et al*., 2025).

RESULTS AND DISCUSSION

The Gas Chromatography and Mass Spectroscopy (GC-MS) of recent are regarded as one of the best and high precision techniques adopted to identify chemical compounds such as the constituents of volatile matter, steroids, terpenoids, ketones, alcohols, hydrocarbons (long and branch chains), etc. The findings of the present study involving the use of GC-MS for the analysis leads to the identification of various compounds from the mycotoxin extract of the solvent extract *C. coccodes*

The chromatogram peaks were unified and were matched with the database of spectrum of known compounds stored in the GC-MS library. Metabolites having significant quantities of greater than 1% (>1%) in terms of percent area identified from the extract of *C. coccodes* as shown in Table 1 are: n-hexadecanoic acid (palmitic acid; RT 13.547 min, 19.02% of total peak area). Other major peaks included methyl hexadecanoate (methyl palmitate; RT 13.181 min, 9.65%), methyl stearate (RT 14.777 min, 6.23%), (E)-9-octadecenoic acid methyl ester (methyl oleate; RT 14.594 min, 7.80%), methyl (E)-13-octadecenoate (RT 14.634 min, 2.44%), cis-9,12-octadecadienoic acid (linoleic acid; RT 14.549 min, 2.37%), and trans-13-octadecenoic acid methyl ester (RT 14.634 min, 2.44%). The free acids oleic acid (cis-9-octadecenoic acid; RT 13.736 min, 3.11%) and linoleic acid were also detected. In the monoterpene region, cis-pulegone (p-menth-8(10)-en-9-ol; RT 14.383 min, 1.25%) was identified. Several long-chain unsaturated alcohols (e.g. 2-methyl-3,13-octadecadien-1-ol, RT ~14.93–15.10 min, total ~12% area) were repeatedly detected as recurring library hits; these likely represent isomers of long-chain terpene alcohols. Minor aromatic compounds such as methyl benzoate (RT 4.604 min, 2.84%) and nitrobenzaldehyde (RT 7.819 min, 7.03%) were present in trace amounts. No known fungal mycotoxins (anthraquinones, tropolones, etc.) were detected among the volatile fraction.

A few additional minor compounds were observed: e.g. 7,11-hexadecadienal ($C_{16}H_{28}O$, aldehyde), various long-chain alcohols (e.g. 2-methyl-3,13-octadecadien-1-ol, $C_{19}H_{36}O$, total ~15% area combined) and saturated hydrocarbons (e.g. cycloeicosane). These have no well-known specific bioactivity and were not referenced in detail. Overall, the extract's profile is dominated by common lipids (fatty acids and methyl esters) and a single

plant-derived monoterpene (pulegone). The relative abundances are approximate; for example, palmitic acid accounted for ~19% of the total ion current, while oleic acid (~3%) and linoleic acid (~2%) were much lower.

The GC-MS profile of *C. coccodes* indicates that its primary metabolites under our growth conditions are typical long-chain fatty acids and their esters. These compounds are ubiquitous in fungal and plant tissues and likely serve general cellular roles (membrane components, energy storage) rather than specialized toxins. Notably, no unique fungal polyketides or alkaloids were detected. The presence of methylated esters suggests that derivatization was effective, but also that many fatty acids were present. It is possible that the extraction and methylation favored detection of lipids, and that non-volatile metabolites (e.g. polyketides) could have been missed. The identified metabolites have been reported to exhibit biological activities relevant to plant–microbe interactions. Palmitic acid (n-hexadecanoate) is known to inhibit virulence factors of Candida and other microbes (Prasath *et al*., 2020). Oleic acid (cis-9-octadecenoate) similarly has antibacterial and antifungal properties (Liszkowska *et al*., 2023). These fatty acids may help the fungus compete with other microorganisms in the soil or on the plant surface, or alternatively they could contribute to host responses. Linoleic acid, another common unsaturated fatty acid, has been implicated in signaling and sporulation in fungi (Ana *et al*.,2022) (though this report did not assess such effects here). Methyl esters like methyl palmitate and methyl stearate often act as semio chemicals (pheromones or repellents) in insects, suggesting possible ecological roles in deterring predators or attracting vectors, though specific activities of these esters in this context are not well documented. The detection of pulegone (p-menth-8(10)-en-9-ol) is interesting because this monoterpene is typically a plant-derived volatile found in mint family essential oils. Pulegone has strong insecticidal and repellent properties (Ainane *et al*., 2023). Its appearance in the GC-MS may reflect either endogenous fungal biosynthesis (there are reports of fungal terpenoid metabolism) or uptake from the potato medium. Regardless, its presence could confer an advantage to the fungus by repelling some insects or microbes. This report note that many of the compounds (palmitic, oleic, linoleic acids and their esters) are also common in tomato and tuber oils. The host-derived components such as chlorogenic acids or tomato glycoalkaloids (e.g. solanine) were not detected by GC-MS, likely because they are non-volatile and not amenable to this GC-MS method.
Importantly, none of the GC-MS-identified metabolites correspond to known mycotoxins or phytotoxins of *Colletotrichum*. Previous LC-MS studies similarly found that *C. coccodes* produces only unspecific metabolites, and reported that compounds like beauvericin or bikaverin detected on inoculated potatoes originated from contaminating *Fusarium* species. Our results are consistent with these findings: we did not observe any fusarium mycotoxins or anthraquinones typical of *Colletotrichum* anthracnose pathogens. Instead, the profile suggests *C. coccodes* relies on general lipid metabolites.

In summary, the GC-MS data imply that *C. coccodes* produces mainly fatty acids, alcohols and esters that have general antimicrobial or insect-repellent properties. These metabolites could contribute to the ecology of the fungus (e.g. suppressing competitors). From a plant pathology perspective, the lack of distinctive phytotoxins suggests that *C. coccodes* pathogenicity may depend more on mechanical damage, enzymatic factors, or host-specific interactions, rather than secreted toxins. Future work could use complementary LC-MS/MS to search for non-volatile metabolites and test the bioactivity of the detected fatty acids against plant pathogens or pests.

CONCLUSION

Gas chromatography–mass spectrometry of *Colletotrichum coccodes* culture extracts revealed a metabolite profile dominated by common fungal fatty acids (palmitic, oleic, linoleic) and their methyl esters, along with one plant-like monoterpene (pulegone). No novel mycotoxins were identified. Many of the major compounds are known to possess antimicrobial or insecticidal activities. This first GC-MS survey of *C. coccodes* chemistry provides a reference for its secondary metabolism and suggests that its contribution to plant disease is unlikely to involve unique toxins. The identified lipids may, however, play a role in fungal fitness or plant–microbe interactions. The present study focused on the identification of mycotoxins produced by *Colletotrichum coccodes* using Gas chromatography and mass spectrometry (GC-MS). Further studies using targeted assays are needed to confirm biological effects of these metabolites in the context of disease.

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