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PROFILING KEY METABOLITES IN SHALLOT UNDER FUSARIUM INFECTION

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SUMMARY

Fusarium disease presents a formidable challenge to shallot (*Allium cepa* L.) production globally, necessitating a profound understanding of the plant's defense mechanisms. Secondary metabolites play a pivotal part in plant-pathogen dynamics, yet their roles against *Fusarium oxysporum* f. sp. cepae (FOC) in shallots remain underexplored. In the presented study, the use of Gas Chromatography-Mass Spectrometry (GC-MS) helped profile the secondary metabolites in six shallot genotypes, i.e., Bima Brebes, Sumenep, Tajuk, Katumi, Biru Lancor, and Maja Cipanas. The analysis revealed substantial variations in the quantity and diversity of compounds between the Fusarium disease infected and non-infected shallot treatments. However, the infected shallots exhibited a more pronounced metabolite profile (168 vs. 95). Notably, the susceptible shallot cultivar Katumi enunciated the highest metabolite production across both conditions. Clustering analysis identified four distinct metabolite clusters for infected and non-infected shallots. Heatmap analysis highlighted elevated levels of cholesterol derivatives, sterol, and linoleic acid in the shallot resistant cultivar Sumenep, positioning these compounds as promising biomarkers and crucial elements in the defense strategy of shallots against Fusarium disease.

Keywords: Shallot (*A. cepa* L.), biomarkers, clustering analysis, Fusarium disease, GC-MS, resistance mechanism, secondary metabolites

Key findings: The study unveiled significant variations in secondary metabolites between the Fusarium disease infected and non-infected shallot (*Allium cepa* L.). However, the infected treatments showed greater diversity and expression (168 compared with 95). Notably, in the shallot resistant cultivar Sumenep, the cholesterol derivatives, sterol, and linoleic acid were considerably higher, indicating their potential as biomarkers for resistance against Fusarium disease.

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INTRODUCTION

Shallot (*Allium cepa* var. *aggregatum* Don, 2n = 16), a member of the Alliaceae family, is a prominent vegetable crop, significantly contributing to vegetable production worldwide. Global vegetable production has surged by 71 percent since 2000, reaching 1.17 billion tons in 2022, with onions and shallots ranking as the second most produced vegetables, at 111 million tons (FAOSTAT, 2024). The versatile applications of shallots in agriculture, culinary, and medicinal practices have led to their cultivation as a valuable commercial crop (Shahrajabian *et al*., 2020a; Pangestuti *et al*. 2022). In Asian diets and herbal medicines, the shallot bulbs' traditional use highlights their cultural importance (Ruksiriwanich *et al*., 2022).

As a key vegetable crop, shallot cultivation also plays a vital role in national and regional economies through agriculture practices (Rahayu *et al*., 2019; Hidayah *et al*., 2023). Research has shown shallots are rich in flavonoid antioxidants like quercetin and kaempferol, which enhance their value for medicinal use (Shahrajabian *et al*., 2020a, b). Additionally, studies have explored the potential of shallots in integrated traditional Chinese medicine to safeguard and improve public health (Shahrajabian *et al*., 2020b).

Unfortunately, Fusarium disease has emerged as a major threat to shallot production in several countries (Degani and Kalman, 2021) and in Indonesia, especially the still-to-be identified resistant cultivars. Fusarium disease has been a longstanding concern for farmers, leading to substantial yield losses, with the losses even occurring up to 100% (Prakoso *et al*., 2016). Given the shallot's continuous cultivation throughout the year, specifically from the dry to rainy seasons, and by providing the host to Fusarium, the disease remains prevalent in the country. Therefore, the Fusarium disease control is challenging as the disease cycle persists (Chaves-Gómez *et al*., 2021; Sangeetha and As, 2021).

The Fusarium wilt management often involves the use of fungicides, causing negative environmental consequences and contributing to the development of fungicide-resistant pathogen strains, which further add to the disease management complexity. In Indonesia, several shallot cultivars, such as, Bima Brebes, Tajuk, Blue Lancor, and Maja Cipanas, are preferences of the farming community for their superior characteristics; although, these cultivars are susceptible to the Fusarium disease (Herlina *et al*., 2021). In contrast, local cultivar Sumenep, while less popular due to a lower yield, exhibited better resistance to the Fusarium disease (Aprilia and Maharijaya, 2020).

Developing sustainable strategies for managing Fusarium wilt, including the use of plant secondary metabolites (PSMs), have shown a crucial role in plant defense mechanism. Plants have innate defense mechanisms, both passive and active, that activate when the plants encounter pathogens. These responses include the production of biochemical compounds inhibiting pathogen growth, such as, phenolic compounds, antioxidants, phytoalexins, and tannins (Bizuneh, 2021; Kaur *et al*., 2022). However, defining specific changes in plant metabolic activities as stress responses can be challenging, as plants often undergo multiple stress factors simultaneously. Nonetheless, plants possess recognition and signaling systems that enable rapid detection of pathogens and the initiation of defense responses. These defenses can include the formation of polymers to block pathogen entry and the synthesis of enzymes to degrade pathogenic cell walls.

Comparative metabolomic analyses have shown shallots to possess higher levels of total flavonoids, alk(en)yl cysteine sulfoxides (ACSOs), and polysaccharides than common onions. Additionally, the LC-Q-TGF-MS analysis of amino acids in short-day and long-day onions and Indonesian shallot landraces revealed several amino acids, polyamines, and organic acids, specifically accumulated in shallots (Abdelrahman *et al*., 2020). In shallot landraces, the higher amino acid profiles suggest this is a metabolic characteristic of shallots. Despite these findings, there remains a paucity of information on the specific secondary metabolites produced by shallots in

response to Fusarium infection. Identifying these compounds is crucial for developing effective disease control strategies.

Understanding the importance of secondary metabolites, the Fusarium-infected shallot cultivars can provide valuable insights into the plant's defense mechanisms and identify the potential targets for breeding programs. This study primarily aimed to analyze the key secondary metabolites present in six Fusarium-infected shallot cultivars and identify the most promising genotypes with biomarkers indicative of resistance to Fusarium disease. These findings contribute to sustainable agricultural practices by pinpointing natural compounds that can enhance shallot resistance to Fusarium wilt. This research also seeks to support the development of Fusarium-resistant shallot cultivars, reduce reliance on chemical fungicides, and promote more effective and environmentally friendly strategies for disease control.

MATERIALS AND METHODS

Experimental site and plant material

The timely study involved six shallot genotypes: geno-1–Katumi (Ktm), geno-2–Tajuk (Tjk), geno-3–Bima Brebes (BmBr), geno-4–Maja Cipanas (MjCp), geno-5–Biru Lancor (BL), and geno-6–Sumenep (Smn). The identification of Katumi genotype as a susceptible variety resulted from the findings of Herlina (2019) [Unpublished], while Sumenep is a recognized resistant genotype, as documented by Aprilia and Maharijaya (2020).

The study commenced at two different sites to optimize the research process. The shallot plants' growing and inoculation with *Fusarium oxysporum* f. sp. *cepae* (FOC) transpired at the ICABIOGRAD (Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development) greenhouse, West Java, Indonesia (6° 34′ 29.54″ S, 106° 47′ 11.73″ E). Collecting the infected bulbs from the six genotypes ensued two weeks after exposure to the FOC-3 isolate. After harvesting, the bulbs' transport to the Laboratory of Health, Jakarta Province, Indonesia (6° 18′ 44.05″ S, 106° 87′ 16.33″ E) continued for further processing.

Plant growth conditions

The shallot bulbs, grown in a greenhouse environment, had the standard growing conditions for onions applied, with temperature at 27 °C–30 °C during the day and 22 °C–26 °C at night. Relative humidity was about 60%–70%, which meet optimal growth conditions, with plants provided a photoperiod of 12 h of light by natural daylight and 12 h of darkness.

Inoculation methodology

Healthy shallot bulbs, selected from each of the six genotypes, served as the source material for both infected and non-infected samples. The shallot genotypes used in this study came from a collection established in a previous study. For each genotype, shallot bulbs' selection relied on uniformity in size and health status to ensure consistency in the analysis. A total of 10 bulbs per genotype were specimens used for the study.

The FOC-3, the Fusarium pathogenic isolate used in this study, came from our collection, with the pathogenicity previously confirmed through a pathogenicity test—cultured on potato dextrose agar (PDA) plates for 10 days at 25 °C to promote conidial production. After this period, the harvest of the conidia proceeded by flooding the culture plates with sterile distilled water, with the resulting suspension filtered through a sterile cheesecloth to remove mycelial fragments. Adjusting the concentration of the conidial suspension to 10^6 conidia/mL used a hemocytometer.

For the inoculation process, dividing the healthy bulbs into two groups occurred: one designated for infection and the other to remain non-infected. The selected bulbs received thorough washing with distilled water to remove any soil or debris before surface-sterilizing with 70% ethanol. After the sterilization, the bulbs reached grouping into

two: one group as inoculated with FOC-3, while the other group as the non-infected control.

The bulbs intended for infection bore submergence in the FOC-3 conidial suspension for 30 min to ensure the pathogen's introduction into the internal tissues of the bulbs. After inoculation, draining the bulbs followed before placing in a humid chamber to facilitate infection. They remained under controlled conditions in the ICABIOGRAD greenhouse to allow the infection to form. In contrast, the control group of bulbs' submergence was in sterile distilled water for 30 min, mirroring the treatment of the infected bulbs, but without exposure to the FOC-3 inoculum. These non-infected bulbs also remained under the same controlled conditions as the infected group to ensure consistency in environmental factors.

Two weeks after exposure to the FOC-3 isolate, the infected bulbs' collection continued to identify as the "infected sample" group. Simultaneously, harvesting the non-infected bulbs served as the "healthy sample" group for the subsequent analysis of secondary metabolites, removing the outer scales. Then, freezing the bulb tissues in liquid nitrogen followed before grinding them to a fine powder using a mortar and pestle. The ground tissue storing at -80 °C remained until further processing at the Laboratory of Health, Jakarta Province, Indonesia. In this facility, sample preparation, extraction, and analysis of secondary metabolites progressed using the Gas Chromatography-Mass Spectrometry (GC-MS), following the procedure described by Kamthan *et al*. (2012). Previous studies also revealed shallot cultivar Sumenep exhibited resistance to FOC, making it a key focus in this study.

All the recorded data tabulation used the Microsoft Excel, and the compound identification in GC-MS analysis utilized the MS-Chemstation G1701-DA with the WILEY spectral library. Further data analysis utilized the principal component analysis (PCA) and hierarchical clustering analysis (HCA) in R-Studio ver. 1.1.442. The PCA classified shallot cultivars and their biochemical compounds, while the HCA, based on the Euclidean distance and the Ward algorithm, visualized the grouping of examined shallot cultivars based on the identified metabolites.

Heatmap generation

The generation of a heatmap using the normalized metabolite data visualized the variation in metabolite expression across the different shallot cultivars. . The data normalization process involved scaling the peak intensities of each metabolite to a uniform scale, allowing for accurate comparisons across samples.

The heatmap construction used the following steps:

Selection of Metabolites: Only metabolites with significant expression (i.e., those showing at least a two-fold change between infected and non-infected samples) were part of the heatmap analysis.

Software and Algorithms: The heatmap generation employed the R-Studio (ver. 1.1.442) with the "pheatmap" package. Hierarchical clustering engaged the Euclidean distance metric and Ward's method to group metabolites and samples based on similarity in their expression profiles.

Data Visualization: The resulting heatmap was color-coded, with red indicating higher expression levels and blue indicating lower expression levels. The dendrograms on the rows and columns represent the clustering of metabolites and cultivars, respectively.

RESULTS

The GC-MS analysis enabled the detection of 168 biochemical compounds in the Fusarium infected bulbs and 95 compounds in non-infected shallot bulbs.

Metabolite profile in non-Infected shallot bulbs

In the analysis of secondary metabolites, the Fusarium non-infected shallot bulbs of six cultivars exhibited considerable variations both

in type and number of metabolites produced (Figure 1). The Bima Brebes cultivar displayed 15 types of secondary metabolites, Tajuk had 14 types, Biru Lancor had 12 types, Maja Cipanas had 17 types, Sumenep had 16 types, and Katumi had the highest number, with 21 types of metabolites. In total, 95 distinct secondary metabolites were notable across all the shallot cultivars.

The predominant primary metabolites identified included palmitic acid (hexadecanoic acid), stearic acid (octadecadienoic acid), and oleic acid. Other identified compounds included methyl sulfone (dimethyl sulfone), tetrahydropyran (cyclohexanone derivatives), propanamide (amide of propanoic acid), furoic acid, acrolein, and ethyl ester. The major secondary metabolites included sitosterol, stigmast (beta-sitosterol), campesterol (ergost-5-en-3-ol), and phenanthrene. Palmitic acid, as a primary metabolite, was the most frequently occurring and abundant metabolite, especially in the shallot cultivar Tajuk. Stearic acid, another primary metabolite, was the second most prevalent organic compound, found in most of the cultivars, with the highest concentration in the shallot cultivar Maja Cipanas.

Metabolite Profile in infected shallot bulbs

In Fusarium-infected bulbs of six shallot genotypes, the analysis of secondary metabolites revealed a more diverse array of metabolites than uninfected bulbs, totaling 168 types of organic compounds identified (Figure 2). The predominant primary metabolite in several cultivars was palmitic acid, while secondary metabolites, such as sterols, were also notably abundant. Specifically, the Bima Brebes cultivar exhibited 32 types of metabolites, with cholesterol (a secondary metabolite) as the most abundant (10.70%). The Tajuk cultivar produced 31 types, with the highest palmitic acid at 11.17%. Biru Lancor showed 30 types, with palmitic acid (17.31%) as the most abundant. Maja Cipanas had 27 types, again with palmitic acid (12.14%) as the most prevalent. The Sumenep cultivar had the fewest types at 18, with the maximum content of sterol (14.94%), a secondary metabolite, indicating its potential role in resistance. Meanwhile, the Katumi cultivar demonstrated 30 types of metabolites, with palmitic acid as the most abundant (17.00%).

Overall, the identified primary metabolites in Fusarium-infected shallot bulbs included palmitic acid (hexadecanoic acid), stearic acid (octadecadienoic acid), linoleic acid, tocopherol, and progesterone. The secondary metabolites detected were cholesterol, phenanthrene, sterols, eicosyne, furfural, and sitosterol. Additionally, the secondary metabolites, such as, tricosane, pentacosane, docosene, and acetate were also evident in almost all samples, albeit with lower peak intensities. Notably, several secondary metabolites exhibited higher intensities in infected bulbs than healthy ones. For instance, while palmitic acid, a primary metabolite, had the highest content in the Tajuk cultivar, it was more generally prominent in Fusarium-infected bulbs. In the Sumenep cultivar, the secondary metabolites octadecadienoic acid, stigmast, and cholesterol occurred at relatively high levels in FOC-infected bulbs.

Secondary metabolite clustering

Clustering analysis continued on 93 secondary metabolites (those >1%). In shallots with Fusarium-infected condition, the cluster plot indicated the secondary metabolites exhibited distinct patterns, grouped into four main clusters (Figure 3). Each cluster represents a different metabolic response, potentially linked to the shallot genotypes' resistance or susceptibility to Fusarium infection. The cluster plot visually represents the grouping of secondary metabolites (Sm) based on their profiles in infected condition across the six shallot genotypes. The plot shows four distinct clusters of secondary metabolites, each represented by different colors (green, blue, yellow, and red). The two axes, Dim1 (28.8%) and Dim2 (16.8%), representing the principal components, capture the major patterns in the metabolite data, with Dim1 explaining the most variation. For the list of secondary metabolite names, please refer to Appendix 1.

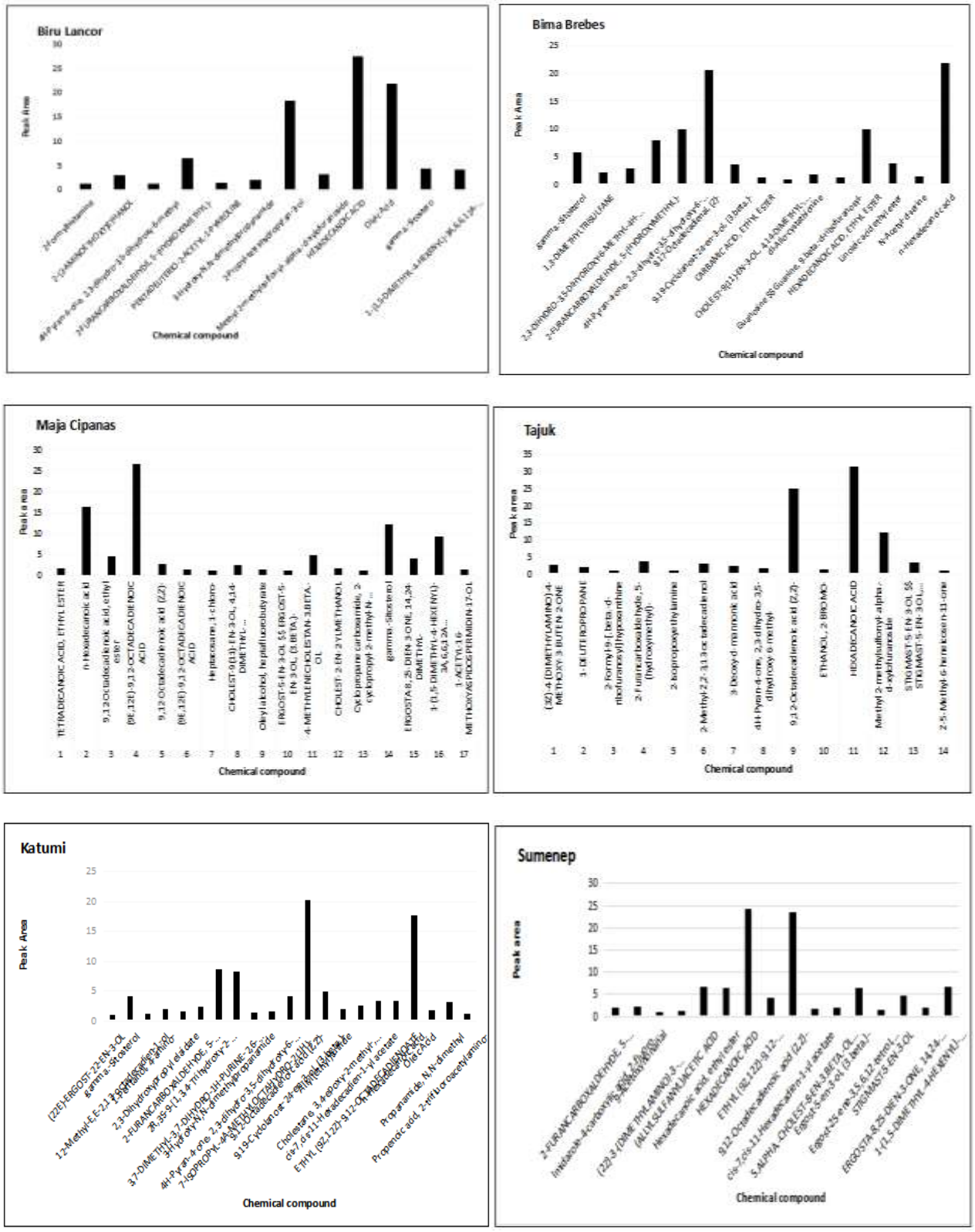

Figure 1. Major secondary metabolites produced by various shallot cultivar bulbs (Biru Lancor, Bima Brebes, Maja Cipanas, Tajuk, Katumi, and Sumenep) with no FOC infection.

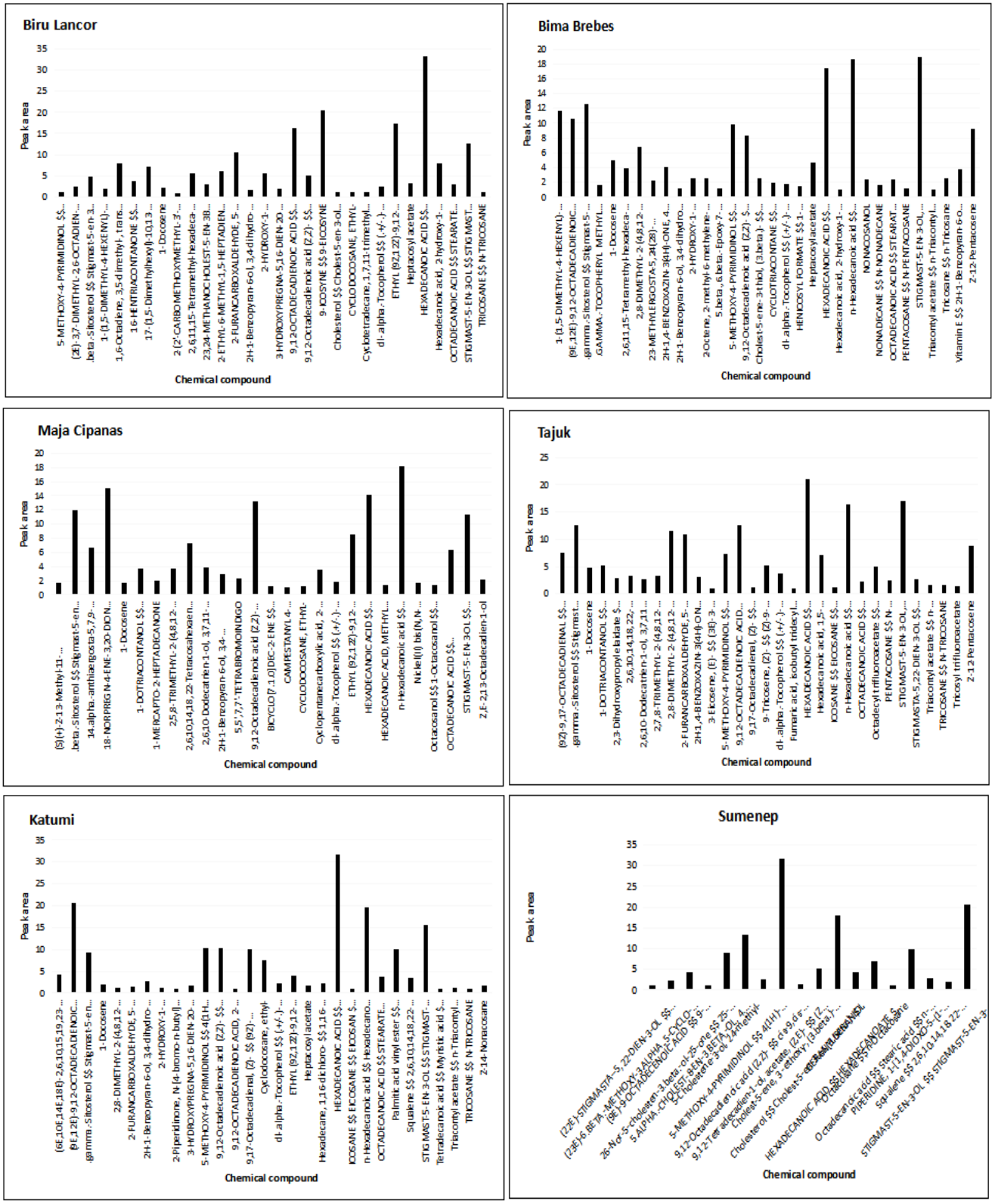

Figure 2. Major secondary metabolites produced by various shallot cultivar bulbs (Biru Lancor, Bima Brebes, Maja Cipanas, Tajuk, Katumi, and Sumenep) infected with FOC.

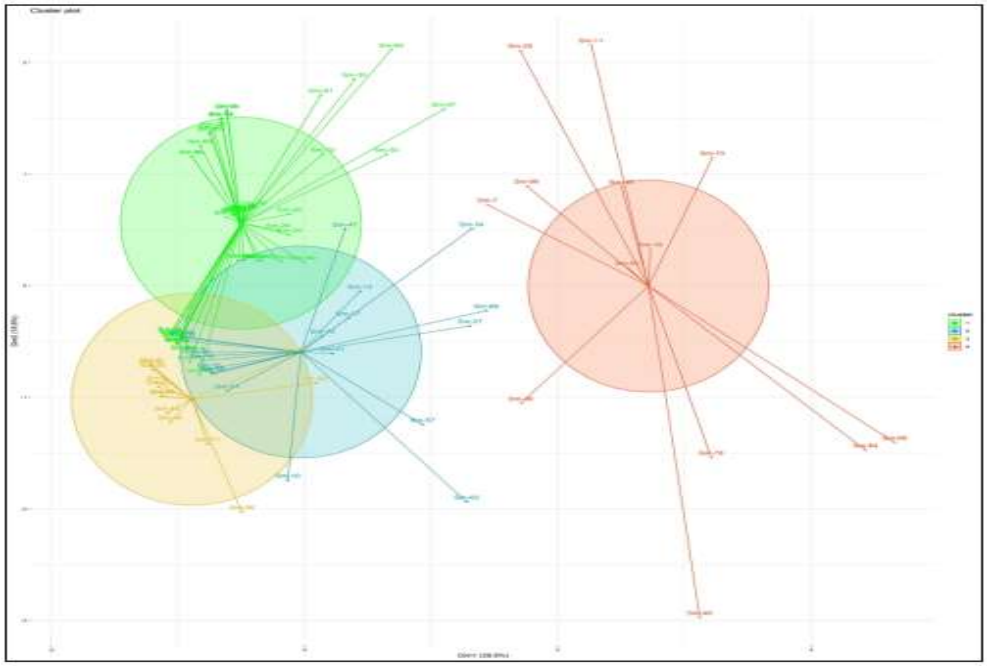

Figure 3. Cluster distribution pattern of secondary metabolites in shallot bulbs infected with FOC.

Cluster 1 (Green) included metabolites, such as, Sm-3, Sm-5, Sm-13, Sm-20, Sm-35, Sm-50, Sm-62, Sm-81, and Sm-92. These metabolites were close in the group, indicating similar profiles under the shallot-infected condition. Shallot genotypes in this cluster likely share similar metabolic responses. Cluster 2 (Blue) contained metabolites—Sm-12, Sm-27, Sm-37, Sm-41, Sm-57, and Sm-66. These metabolites also grouped closely, suggesting a related expression pattern under Fusarium infection. This cluster was distinct from the green cluster, implying a different metabolic response.

Cluster 3 (Yellow) comprised metabolites, such as, Sm-1, Sm-6, Sm-9, Sm-14, Sm-17, Sm-45, and Sm-50. In this cluster, the metabolites exhibited unique profiles, suggesting a different metabolic response compared with the other clusters. Cluster 4 (Red) contained metabolites Sm-7, Sm-10, Sm-11, Sm-19, Sm-29, Sm-38, Sm-46, Sm-73, Sm-78, and Sm-84. These metabolites displayed a very distinct pattern, separate from the other three clusters, indicating a unique metabolic response to Fusarium infection.

The clustering in non-infected treatments showed different groupings of metabolites compared with the infected condition (Figure 4). Cluster 1 (Green) included metabolites, such as, Sm-4, Sm-24, Sm-25, Sm-32, Sm-36, Sm-40, Sm-55, Sm-65, and Sm-70, which were closely grouped, indicating similar profiles under the non-infected condition. Cluster 2 (Blue) had metabolites Sm-15, Sm-19, Sm-28, Sm-50, Sm-60, Sm-77, and Sm-82, also grouped tightly, suggesting a related expression pattern under a shallot non-infected condition.

Cluster 3 (Yellow) consisted of metabolites Sm-3, Sm-6, Sm-10, Sm-14, Sm-23, Sm-27, and Sm-33. These metabolites exhibited distinct profiles versus other clusters, suggesting a different metabolic response. Cluster 4 (Red) contained metabolites Sm-7, Sm-9, Sm-13, Sm-17, Sm-29, Sm-31, Sm-37, Sm-43, Sm-46, and Sm-52. These metabolites displayed a unique pattern, separated from the other three clusters. Overall, metabolites like Sm-15, Sm-19, Sm-28, Sm-50, Sm-60, and Sm-77 in cluster one, signified a different metabolic profile under the non-infected compared with the infected condition.

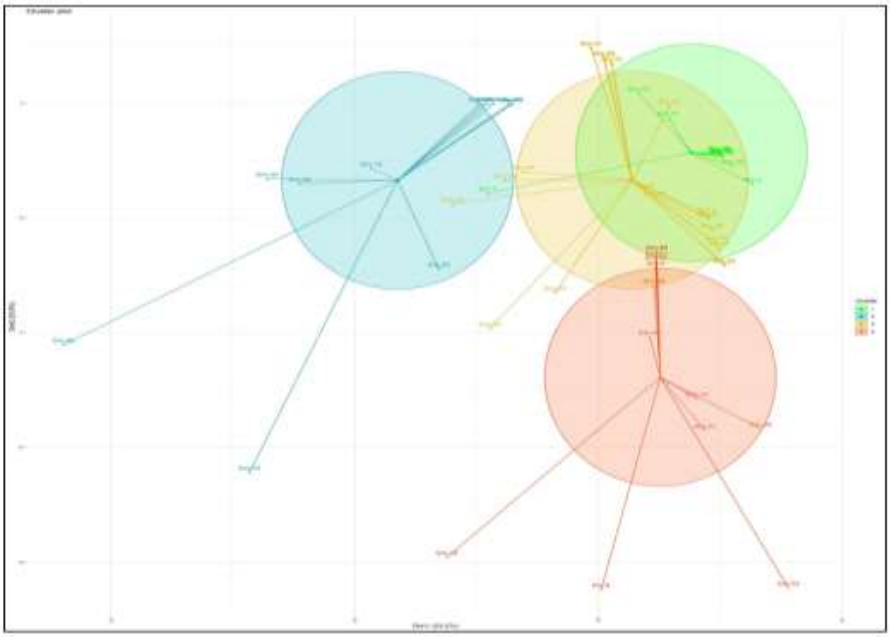

Figure 4. Cluster distribution pattern of secondary metabolites in shallot bulbs non-infected with FOC.

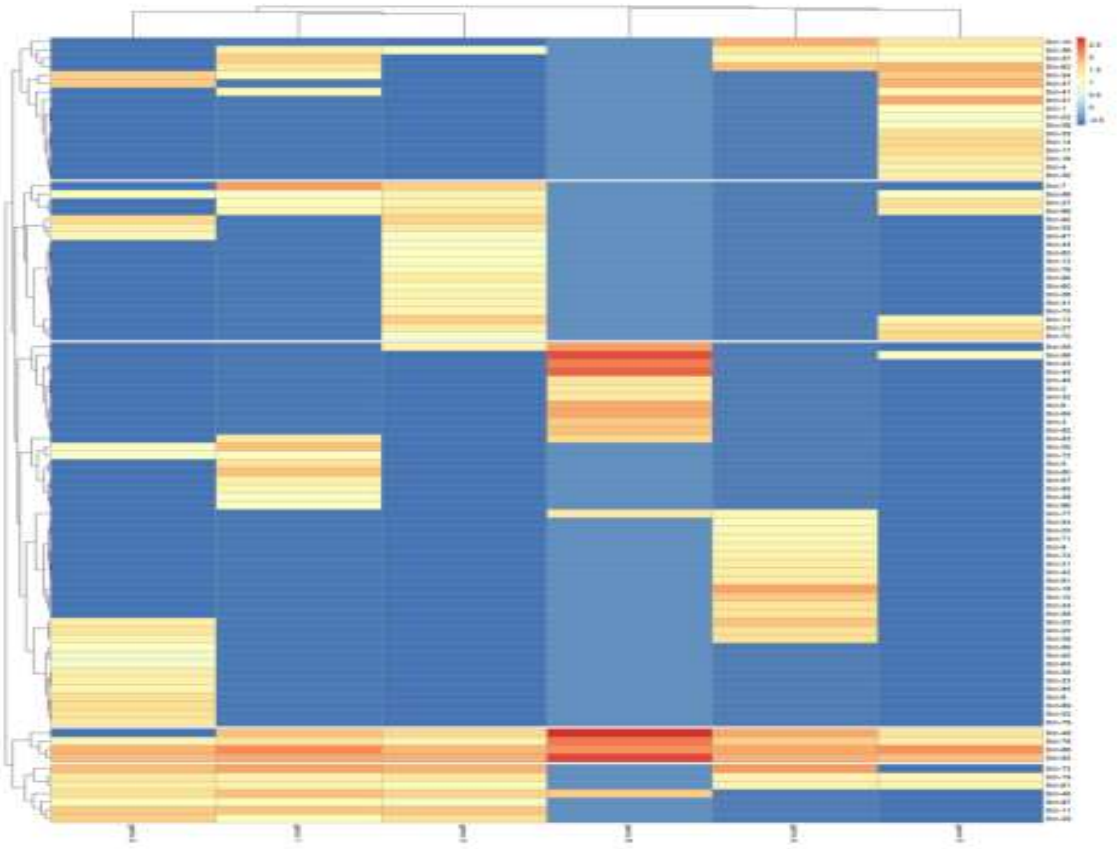

Figure 5. Heatmap distribution pattern of secondary metabolites in shallot bulbs infected with FOC.
Note: geno-1: Katumi (Ktm), geno-2: Tajuk (Tjk), geno-3: Bima Brebes (BmBr), geno-4: Maja Cipanas (MjCp), geno-5: Biru Lancor (BL), and geno-6: Sumenep (Smn).

Heatmap analysis of infected shallot

Based on the heatmap analysis (Figure 5), the metabolites Sm-2, Sm-3, Sm-6, Sm-32, Sm-43, Sm-45, Sm-49, Sm-55, Sm-56, Sm-64, and Sm-82 exhibited minimal expression levels in susceptible and moderately resistant genotypes. However, they appeared at a higher expression level in the shallot resistant genotype (Sumenep). Notably, the metabolites Sm-43, Sm-45, Sm-56, and Sm-84 showed significantly superior content in cultivar Sumenep than other shallot genotypes. Specifically, metabolites Sm-43, Sm-45, and Sm-56 were present at higher levels only in the resistant cultivar Sumenep, authenticating them as strong candidates for biomarkers of resistance. These metabolites could directly participate in the resistance mechanisms against Fusarium infection.

Metabolites, viz., Sm-48, Sm-78, Sm-83, and Sm-84 exhibited more complex distribution patterns. Although, they still showed a higher level in the shallot resistant genotype Sumenep. Noteworthily, metabolite Sm-84 was consistently highly expressed across all the genotypes, with the optimum level in cultivar Sumenep, suggesting its crucial role in resistance. Metabolite Sm-48 displayed variable expression among the shallot genotypes, while showing the maximum concentration in cultivar Sumenep, indicating its potential involvement in resistance mechanisms. Both metabolites Sm-84 and Sm-48 likely play crucial roles in the resistance of shallots against Fusarium disease.

DISCUSSION

As previously mentioned, the metabolites Sm-43, Sm-45, and Sm-56 appeared with a higher concentration in the shallot resistant cultivar Sumenep, identified as strong biomarkers of resistance. These metabolites were all cholesterol derivatives, playing significant roles in plant physiology and defense mechanisms. Specifically, Sm-43 (5.ALPHA.-CHOLEST-8-EN-3.BETA.-OL, 4.ALPHA., 14-DIMETHYL-CHOLest-8-EN-3-OL, 4, 14-DIMETHYL-, (3.BETA., 4.ALPHA., 5.ALPHA.)-24-DEMETHYOBTUSIFOLIOL, 29-NORLANOST-8-EN-3.BETA.-OL), Sm-45 (5-Cholestene-3-ol, 24-methyl-), and Sm-56 (Cholesterol, Cholest-5-en-3-ol (3.beta.)-, (-)-Cholesterol, Cholest-5-en-3.beta.-ol) proved crucial in plant defense mechanisms.

Phytosterols like brassicasterol, sitosterol, chalinasterol, and campesterol imply a plant's ability to synthesize essential sterols for various physiological functions (Kohlbach *et al*., 2021). These sterols are key components of plant cell membranes, contributing to structural integrity and functionality. Beyond structural roles, these phytosterols act as signaling molecules, regulating plant growth, stress responses, and defense mechanisms. These organic compounds also influence cell membrane properties, such as, fluidity and permeability, and regulate the membrane-bound enzymes essential for plant development and various physiological processes (Kohlbach *et al*., 2021).

Notably, metabolites Sm-84 and Sm-48 were consistently higher across all shallot genotypes. However, they gave the highest concentration in resistant cultivar Sumenep, suggesting a crucial role in the resistance mechanism of shallots against Fusarium disease. Metabolite Sm-84 is stigmasterol (stigmast-5-en-3-ol, [3β,24S]), a major plant sterol integral to plant physiology and defense mechanisms. Stigmasterol, along with other plant sterols like β-sitosterol and campesterol, are essential components of plant membranes (Cabianca *et al*., 2021). These sterols regulate membrane fluidity, integrity, and permeability, and are vital for various physiological functions (Sarkar *et al*., 2021). Stigmasterol significantly influences cell differentiation, proliferation, and stress responses. Its accumulation during plant development, as observed in *Arachis hypogaea* seeds, correlates to physiological maturation and stress resistance. Past research reported stigmasterol biosynthesis occurs from the control of specific genes, providing insights into its roles in plant development and stress responses (Aboobucker and Suza, 2019).

Stigmasterol participation has proven to enhance plant resistance to biotic and abiotic stresses, suggesting its involvement in systemic acquired resistance (SAR), a defense

mechanism providing long-lasting protection against a wide range of pathogens (Huang *et al*., 2022). Past studies also explored the molecular mechanisms underlying stigmasterol's effects, showing it can regulate reactive oxygen species (ROS) levels (Bakrim *et al*., 2022). Additionally, stigmasterol's role in plant resistance connects to its regulation of sterol synthesis and maintenance of plasma membrane stability, which are crucial for defense responses. The alteration of stigmasterol levels in response to stresses and pathogens further supports its importance in plant metabolism and defense mechanisms (Gutiérrez-García *et al*., 2021).

Metabolite Sm-48 (9,12-Octadecadienoic acid [Z,Z], cis-9,cis-12-Octadecadienoic acid, cis,cis-Linoleic acid, Grape seed oil) is linoleic acid, an essential fatty acid with a considerable role in plant defense mechanisms. Linoleic acid acts as a precursor to jasmonic acid, a key signaling compound involved in plant defense and development (Zhang *et al*., 2023). Fatty acids and their derivatives enhance stress tolerance by participating in various plant defense pathways. Linoleic acid, a crucial polyunsaturated fatty acid, is the most common in plant oils and occurs at a higher level in commercial oils, often exceeding 50%, which is necessary for normal plant growth (Geng *et al*., 2020). It also plays a remarkable role in lipid peroxidation, with the oxidation products of polyunsaturated fatty acids by lipoxygenase (LOX), regulating growth, development, and defense responses to stress (Holková *et al*., 2019). Additionally, linoleic acid positively regulates plant defense against pathogens, i.e., *Verticillium dahliae,* by modulating fatty acid accumulation and the jasmonic acid signaling pathway (Zhu *et al*., 2021). Plant oxylipins, derived from C18 polyunsaturated fatty acids (linoleic acid), showed their involvement in pathogen-specific defense mechanisms against fungal infections (An *et al*., 2019).

Linoleic acid also interlinked with nematicidal activity, as demonstrated in the *Holigarna caustica* fruit, where its presence effectively controlled the nematodes (Panda *et al*., 2020). Additionally, linoleic acid has revealed associations with inhibiting the activity of certain Lactobacillus strains by affecting their cell membranes and normal metabolism, showing its diverse effects (Lv *et al*., 2020). These findings underscore the multifaceted roles of linoleic acid in plant defense and stress responses. As an essential fatty acid, linoleic acid contributes to producing signaling compounds, regulates lipid metabolism, stimulates defense response genes, and enhances stress tolerance. Therefore, its involvement in various pathways highlights its significance in plant immunity and adaptation to environmental challenges.

Based on the above results, determining which metabolites to choose as markers requires careful consideration. Candidate biomarkers are the secondary metabolites providing a specific physiological state, such as, disease resistance. These metabolites can diagnose resistance without necessarily revealing their biological role in the resistance mechanism. Such metabolites emerged with higher concentrations in shallot resistant genotypes than susceptible and moderately resistant ones, and can benefit the screening and selection of resistant genotypes based on their ratios. These metabolites consistently showed higher levels in resistant plants across various conditions (Holková *et al*., 2019).

The differences in metabolite concentrations between resistant and moderately resistant genotypes suggest a gradient in the defense response. In the moderately resistant genotypes, the concentration of key metabolites may be sufficient to confer a partial defense, leading to a reduced but not complete resistance. It indicates these genotypes can initiate defense mechanisms, but perhaps, not as robustly as the fully resistant genotype. The moderate resistance observed could be due to a combination of factors, including the presence of these metabolites at lower concentrations, the involvement of other defense pathways, or genetic factors partially activating the same resistance mechanisms. It is also possible in moderately resistant genotypes, additional stress-response mechanisms are at play, which was not the focus of this study.

Considering these criteria, the metabolites Sm-43, Sm-45, Sm-56, and Sm-84 were the samples identified as candidate biomarkers due to their significantly higher abundance in the shallot resistant genotype Sumenep than susceptible ones. Their consistent presence with a higher concentration in resistant plants makes them useful for screening (Zhang *et al*., 2023). These metabolites serve as excellent biomarkers for identifying resistant genotypes, and further research, such as, biochemical assays and gene expression studies, is necessary to determine their direct role in the resistance mechanisms.

Secondary metabolites involved in resistance mechanisms and actively participated in the biochemical and physiological processes conferring resistance to crop plants. These metabolites are integral to the plant's defense system, directly participating in defense pathways, such as, antimicrobial activity, signaling, and strengthening plant cell walls. Said metabolites contribute to known defense mechanisms, including producing reactive oxygen species, inhibiting pathogen growth, and signaling other defense responses (Zhu *et al*., 2021). The identification of these metabolites resulted from the pathway analysis, as part of critical defense-related pathways.

Based on these criteria, metabolites Sm-48 and Sm-78 showed variable ratios among the shallot genotypes; however, were the highest in the resistant genotype Sumenep. These metabolites could contribute to specific biochemical pathways to enhance the resistance, such as, antimicrobial activity and signaling pathways to activate other defense responses against the Fusarium disease. Understanding their role can lead to the development of targeted breeding strategies through molecular breeding and can enhance resistance by manipulating their biosynthetic pathways through genetic engineering (Huang *et al*., 2022).

The study on the secondary metabolites of shallots infected with *Fusarium oxysporum* f. sp. cepae (FOC) revealed significant differences in metabolite profiles between the infected and non-infected group of bulbs, as well as, among the different shallot genotypes. The shallot resistant genotype Sumenep exhibited a unique profile of metabolites, including cholesterol derivatives, sterol derivatives, and linoleic acid. As linoleic acid is a primary metabolite involved in basic plant metabolism, the cholesterol and sterol derivatives are secondary metabolites known for their roles in plant defense mechanisms. These findings highlight the complex and varied production of metabolites in shallot genotypes in response to FOC infection and provide insights into potential targets for breeding programs aimed at enhancing resistance to Fusarium disease. Further research focusing on the specific roles of these metabolites in plant-pathogen interactions could lead to developing more effective strategies for disease management in the shallot crop (Nemtinov *et al*., 2021; Maharijaya *et al*., 2023).

CONCLUSONS

The secondary metabolite profiling of non-infected and Fusarium-infected shallot bulbs in six cultivars revealed significant variations, and the infected bulbs exhibited the highest diversity. Key metabolites, such as, Sm-43 (5.ALPHA.-CHOLEST-8-EN-3.BETA.-OL, 4.ALPHA., 14-DIMETHYL-CHOLest-8-EN-3-OL, 4, 14-DIMETHYL-, (3.BETA., 4.ALPHA., 5.ALPHA.)-24-DEMETHYOBTUSIFOLIOL, 29-NORLANOST-8-EN-3.BETA.-OL), Sm-45 (5-Cholestene-3-ol, 24-methyl-), Sm-56 (Cholesterol, Cholest-5-en-3-ol (3.beta.)-, (-)-Cholesterol, Cholest-5-en-3.beta.-ol), and Sm-84 (stigmast-5-en-3-ol, (3β,24S), were remarkable with higher concentrations in the resistant genotype Sumenep, confirming them as strong candidates for biomarkers of resistance. Clustering and heatmap analyses further highlighted distinct metabolic responses, underscoring the importance of these metabolites in plant defense mechanisms. The promising findings provide valuable insights for breeding programs aimed at enhancing Fusarium resistance in shallots.

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Appendix -1. List of secondary metabolites on graph.

Code	Infected	NON-Infected
Sm-1	5-METHOXY-4-PYRIMIDINOL $$ 4(1H)-PYRIMIDINONE, 5-METHOXY- $$ 4-PYRIMIDINOL, 5-METHOXY- $$ 4-HYDROXY-5-METHOXYPYRIMIDINE	(22E)-ERGOST-22-EN-3-OL
Sm-2	(22E)-STIGMASTA--5,22-DIEN-3-OL $$ STIGMASTA-5-22-DIEN-3-OL (3.BETA.22E)-$$ 922E)-STIGMASTA-5,22-DIEN-3.BETA.-OL$$(22E,24S)-24 ETHYL-CHOLESTA-5,22-DIEN-3.BETA-OL	(2Z)-3-(DIMETHYLAMINO)-3-ETHOXY-2-PROPENAL
Sm-3	(23E)-6.BETA.-METHOXY-3.ALPHA.,5-CYCLO-5.ALPHA.-CHOLEST-23-ENE	(3Z)-4-(DIMETHYLAMINO)-4-METHOXY-3-BUTEN-2-ONE
Sm-4	(2E)-3,7-DIMETHYL-2,6-OCTADIEN-1-OL $$ 2,6-OCTADIEN-1-OL, 3,7-DIMETHYL-, (E)- $$ 3,7-DIMETHYLOCTA-2,6-DIEN-1-OL $$ (E)-3,7-DIMETHYL-2,6-OCTADIEN-1-OL	(9E,12E)-9,12-OCTADECADIENOIC ACID
Sm-5	(6E,10E,14E,18E)-2,6,10,15,19,23-HEXAMETHYL-2,6,10,14,18,22-TETRACOSAHEXAENE $$ 2,6,10,14,18,22-TETRACOSAHEXAENE, 2,6,10,15,19,23-HEXAMETHYL- $$ 2,6,10,14,18,22,-TETRACOSAHEXAEN, 2,6,10,15,19,23-HEXAMETHYL- $$ SKVALEN	(ALLYLSULFANYL)ACETIC ACID
Sm-6	(9E)-9-OCTADECENOIC ACID $$ 9-OCTADECENOIC ACID (Z)- $$ OCTADEC-9-ENOIC ACID $$ (9Z)-9-OCTADECENOIC ACID	(Z,Z)-3,13-OCTADECADIEN-1-OL
Sm-7	(9E,12E)-9,12-OCTADECADIENOIC ACID $$ 9,12-OCTADECADIENOIC ACID (Z,Z)- $$ (9Z,12Z)-OCTADECADIENOIC ACID $$ (Z,Z)-9,12-OCTADECADIENOIC ACID	alpha.-D-Mannopyranoside, methyl 2,3,4,6-tetra-O-methyl-
Sm-8	(9Z)-9,17-OCTADECADIENAL $$ 9,17-OCTADECADIENAL, (Z)- $$ 9,17-OCTADECADIENAL (Z) $$ CIS,CIS-OCTADECA-9,17-DIENAL	.gamma.-Sitostero
Sm-9	(S)(+)-Z-13-Methyl-11-pentadecen-1-ol acetate	PHENANTHREN-7-OL
Sm-10	.beta.-Sitosterol $$ Stigmast-5-en-3-ol, (3.beta.)- $$ Stigmast-5-en-3.beta.-ol $$ .alpha.-Dihydrofucosterol	1,2-O-(1-METHYLETHYLIDENE)HEXOFURANOSE
Sm-11	.gamma.-Sitosterol $$ Stigmast-5-en-3-ol, (3.beta.,24S)- $$ Stigmast-5-en-3.beta.-ol, (24S)- $$ Clionasterol	1,3-DIMETHYLTRISULFANE
Sm-12	.GAMMA.-TOCOPHERYL METHYL ETHER	1,4-BUTANE-1,1,4,4-D4-DIAMINE
Sm-13	1-(1,5-DIMETHYL-4-HEXENYL)-3A,6,6,12A-TETRAMETHYLTETRADECAHYDRO-1H-CYCLOPENTA[A]CYCLOPROPA[E]PHENANTHREN-7-OL $$ 9,19-CYCLOLANOST-24-EN-3-OL, (3.BETA.)- $$ 9,19-CYCLO-9.BETA.-LANOST-24-EN-3.BETA.-OL $$ 9,19-CYCLO-9BETA-LANOST-24-EN-3BETA-OL	12-Methyl-E,E-2,13-octadecadien-1-ol
Sm-14	1,6-Octadiene, 3,5-dimethyl-, trans- $$ 1,6-Octadiene, 3,5-dimethyl-, cis- $$ (6E)-3,5-Dimethyl-1,6-octadiene #	16-Nitrobicyclo[10.4.0]hexadecan-1-ol-13-one
Sm-15	14.alpha.-anthiaergosta-5,7,9-trien-3-ol $$ Anthiaergostan-5,7,9-trien-3-ol $$ Anthraergostatrien-3-ol $$ 3a,6-Dimethyl-3-(1,4,5-trimethylhexyl)-2,3,3a,4,5,7,8,9,10,11b-decahydro-1H-cyclopenta[a]anthracen-8-ol #	1-ACETYL-16-METHOXYASPIDOSPERMIDIN-17-OL
Sm-16	16-HENTRIACONTANONE $$ HENTRIACONTAN-16-ONE $$ 16-HEBTRIACONTANONE $$ DIPENTADECYL KETONE	1-DEUTEROPROPANE
Sm-17	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	1-Pentanol, 4-amino
Sm-18	18-NORPREGN-4-ENE-3,20-DIONE, 13-ETHYL- $$ 18-METHYL-PROGESTERONE	2-(2-AMINOETHOXY)ETHANOL
Sm-19	1-Docosene	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE
Sm-20	1-DOTRIACONTANOL $$ DOTRIACONTANOL $$ N-DOTRIACONTANOL	2,3-Dihydroxypropyl elaidate
Sm-21	1-MERCAPTO-2-HEPTADECANONE	2,4,5-TRIMETHYL-1,3-DIOXOLANE
Sm-22	2-(2'-CARBOMETHOXYMETHYL-3'-OXOBUTYL)-1,4-DIHYDROXY-5-METHOXYANTHRAQUINONE	2-Formyl-9-[.beta.-d-ribofuranosyl]hypoxanthine
Sm-23	2,3-Dihydroxypropyl elaidate $$ 2,3-Dihydroxypropyl (9E)-9-octadecenoate #	2-Formylhistamine
Sm-24	2,5,8-TRIMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)-6-CHROMANOL $$ 2H-1-BENZOPYRAN-6-OL, 3,4-DIHYDRO-2,5,8-TRIMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)- $$ .BETA.-TOCOPHEROL $$ .BETA.-TOKOFEROL	2-FURANCARBOXALDEHYDE, 5-(HYDROXYMETHYL)
Sm-25	2,6,10,14,18,22,-TETRACOSAHEXAEN, 2,6,10,15,19,23-HEXAMETHYL- $$ 2,6,10,15,19,23-HEXAMETHYL-	2-HYDROXYCYCLOPENTADECANONE

	2,6,10,14,18,22-TETRACOSAHEXAENE	
Sm-26	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)- $$ (E,E)-Farnesol $$ (2E,6E)-Farnesol $$ All-trans-Farnesol	2-Isopropoxyethylamine
Sm-27	2,6,11,15-Tetramethyl-hexadeca-2,6,8,10,14-pentaene $$ (6E,8E,10E)-2,6,11,15-Tetramethyl-2,6,8,10,14-hexadecapentaene #	2-Methyl-Z,Z-3,13-octadecadienol
Sm-28	2,7,8-TRIMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)-6-CHROMANOL $$ 2H-1-BENZOPYRAN-6-OL, 3,4-DIHYDRO-2,7,8-TRIMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)- $$ .GAMMA.-TOCOPHEROL $$ .GAMMA.-TOKOFEROL	2-Propyl-tetrahydropyran-3-ol
Sm-29	2,8-DIMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)-6-CHROMANOL $$ (2R(2R*(4R*,8R*)))-3,4-DIHYDRO-2,8-DIMETHYL-2-(4,8,12-TRIMETHYLTRIDECYL)-2H-BENZOPYRAN-6-OL $$ .DELTA.-TOCOPHEROL $$ .GAMMA.-TOCOPHEROL	2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl]guanine
Sm-30	23,24-METHANOCHOLEST-5-EN-3B-OL	3(2H)-Furanone, 2-hexyl-5-methyl-
Sm-31	23-METHYLERGOSTA-5,24(28)-DIEN-3.BETA.-OL A	3,5-DIETHYL-1,2,4-TRITHIOLANE
Sm-32	26-Nor-5-cholesten-3.beta.-ol-25-one $$ 25-Norcholesterol, 25-oxo-	3,5-DIHYDROXY-6-METHYL-2,3-DIHYDRO-4H-PYRAN-4-ONE
Sm-33	2-ETHYL-6-METHYL-1,5-HEPTADIENE $$ 1,5-HEPTADIENE, 2-ETHYL-6-METHYL- $$ 2-METHYL 6-METHYLENE 2-OCTENE $$ 2-OCTENE, 2-METHYL-6-METHYLENE-	3,7-DIMETHYL-3,7-DIHYDRO-1H-PURINE-2,6-DIONE
Sm-34	2-FURANCARBOXALDEHYDE, 5-(HYDROXYMETHYL)- $$ 2-FURALDEHYDE, 5- (HYDROXYMETHYL)- $$ 2-FURALDEHYDE, 5-(HYDROXYMETHYL)- $$ 2-FURANCARBOXALDEHYDE, 5- (HYDROXYMETHYL)-	3,7-NONADIEN-1-ONE, 1-(6,6-DIMETHYLBICYCLO[3.1.1]HEPT-2-EN-2-YL)-4,8-DIMETHYL-, (E)-
Sm-35	2H-1,4-BENZOXAZIN-3(4H)-ONE, 4-HYDROXY-2,7,8-TRIMETHOXY- $$ 4-HYDROXY-2,7,8-TRIMETHOXY-2H-1,4-BENZOXAZIN-3-ONE	3-Deoxy-d-mannoic lactone
Sm-36	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]- $$ .delta.-Tocopherol $$ 3,4-Dihydro-2,8-dimethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-ol $$ 8-Methyltocol	3-Deoxy-d-mannonic acid
Sm-37	2-HYDROXY-1-(HYDROXYMETHYL)ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE $$ 9,12-OCTADECADIENOIC ACID (Z,Z)-, 2-HYDROXY-1-(HYDROXYMETHYL)ETHYL ESTER $$ .BETA.-MONOLINOLEIN $$ BETA-MONOLINOLEIN	3-Hydroxy-N,N-dimethylpropanamide
Sm-38	2-Octene, 2-methyl-6-methylene- $$ 2-Methyl 6-methylene 2-octene $$ 2-Ethyl-6-methyl-1,5-heptadiene #	3-PENTANONE, DIMETHYLHYDRAZONE
Sm-39	2-Piperidinone, N-[4-bromo-n-butyl]- $$ 1-(4-Bromobutyl)-2-piperidinone #	4A,7-METHANO-4AH-NAPHTH[1,8A-B]OXIRENE, OCTAHYDRO-4,4,8,8-TETRAMETHYL-
Sm-40	3-Eicosene, (E)- $$ (3E)-3-Icosene #	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl
Sm-41	3-HYDROXYPREGNA-5,16-DIEN-20-ONE $$ PREGNA-5,16-DIEN-20-ONE, 3-HYDROXY-, (3.BETA.)- $$ .DELTA.16-PREGNENOLONE $$ 16-DEHYDROPREGNENOLONE	4-METHYLENECHOLESTAN-3.BETA.-OL
Sm-42	5,5',7,7'-TETRABROMOINDIGO	5.ALPHA.-CHOLEST-8-EN-3.BETA.-OL, 4.ALPHA.,14-DIMETHYL-
Sm-43	5.ALPHA.-CHOLEST-8-EN-3.BETA.-OL, 4. ALPHA., 14-DIMETHYL- $$ CHOLEST-8-EN-3-OL, 4, 14-DIMETHYL-, (3.BETA., 4.ALPHA., 5.ALPHA.)-$$ 24-DEMETHYOBTUSIFOLIOL $$ 29-NORLANOST-8-EN-3.BETA.-OL	5-METHOXY-4-PYRIMIDINOL
Sm-44	5.beta.,6.beta.-Epoxy-7-bromocholestan-3-one $$ 6-Bromo-9-(1,5-dimethylhexyl)-9a,11b-dimethyltetradecahydrocyclopenta[1,2]phenanthro[8a,9-b]oxiren-3(4H)-one #	6,6,10-TRIMETHYL-1-PHENYLTHIOSPIRO(3.6)DEC-1-ENE
Sm-45	5-Cholestene-3-ol, 24-methyl-	7-Ergostenol
Sm-46	5-METHOXY-4-PYRIMIDINOL $$ 4(1H)-PYRIMIDINONE, 5-METHOXY- $$ 4-PYRIMIDINOL, 5-METHOXY- $$ 4-HYDROXY-5-METHOXYPYRIMIDINE	7-ISOPROPYL-4A-METHYLOCTAHYDRO-2(1H)-NAPHTHALENONE
Sm-47	9,12-OCTADECADIENOIC ACID $$ LINOLSAEURE	9,12-Octadecadienoic acid (Z,Z)
Sm-48	9,12-Octadecadienoic acid (Z,Z)- $$ cis-9,cis-12-Octadecadienoic acid $$ cis,cis-Linoleic acid $$ Grape seed oil	9,12-Octadecadienoic acid, ethyl ester
Sm-49	9,12-Tetradecadien-1-ol, acetate, (Z,E)- $$ (Z)-9-(E)-12-Tetradecadien-1-ol acetate $$ Z,E-9,12-Tetradecadien-1-yl acetate $$ Z,E-9,12-Tetradecadien-1-ol acetate	9,17-Octadecadienal, (Z)-
Sm-50	9,17-Octadecadienal, (Z)- $$ (9Z)-9,17-Octadecadienal #	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-
Sm-51	9-ICOSYNE $$ 9-EICOSYNE	9-Acetoxynonanal
Sm-52	9-Tricosene, (Z)- $$ (Z)-9-Tricosene $$ cis-9-Tricosene $$	9-OCTADECYNE

	Muscalure	
Sm-53	BICYCLO[7.1.0]DEC-2-ENE $$ BICYCLO[[7.1.0]DEC-2-ENE	ACETIC ACID, OXO-
Sm-54	CAMPESTANYL 4-ACETYLFERULATE"	Allyl(ethyl)sulfide
Sm-55	Cholest-5-ene, 3 -ethoxy-,(3.beta.)-$$bCholest-5-ene, 3.beta.-ethoxy-$$ Cholesterin ethyl ether $$ Cholesteryl ethyl ether	CARBAMIC ACID, ETHYL ESTER
Sm-56	Cholesterol $$ Cholest-5-en-3-ol (3.beta.)- $$ (-)-Cholesterol $$ Cholest-5-en-3.beta.-ol	CHOLEST-2-EN-2-YLMETHANOL
Sm-57	CYCLODOCOSANE, ETHYL-	CHOLEST-9(11)-EN-3-OL, 4,14-DIMETHYL-, (3.BETA.,4.ALPHA.,5.ALPHA.)-
Sm-58	Cyclopentanecarboxylic acid, 2-bromo-4-fluorophenyl ester	Cholestan-3-one, 4,4-dimethyl-, (5.alpha.)-
Sm-59	Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)- $$ Cyclotetradecane, 4-isopropyl-1,7,11-trimethyl- $$ Cembrane $$ Cembrene, octahydro-	Cholestane, 3,4-epoxy-2-methyl-, (2.alpha.,3.alpha.,4.alpha.,5.alpha.)-
Sm-60	CYCLOTRIACONTANE $$ CYCLOTRIACONTAN	cis-7,cis-11-Hexadecadien-1-yl acetate
Sm-61	dl-.alpha.-Tocopherol $$ (.+/-.)-.alpha.-Tocopherol $$ Vitamin E $$ 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-	Cyclododecanone, 2-methylene-
Sm-62	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE $$ 9,12-OCTADECADIENOIC ACID (9Z,12Z)-, ETHYL ESTER $$ 9,12-OCTADECADIENOIC ACID (Z,Z)-, ETHYL ESTER $$ 9,12-OCTADECADIENOIC ACID ETHYL ESTER	Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-
Sm-63	Fumaric acid, isobutyl tridecyl ester	d-Glycero-d-galacto-heptose
Sm-64	GERANYLGERANIOL	dl-Allo-cystathionine
Sm-65	HENICOSYL FORMATE $$ 1-HENEICOSYL FORMATE	E-8-Methyl-7-dodecen-1-ol acetate
Sm-66	Heptacosyl acetate	Ergost-25-ene-3,5,6,12-tetrol, (3.beta.,5.alpha.,6.beta.,12.beta.)-
Sm-67	Hexadecane, 1,16-dichloro- $$ 1,16-Dichlorohexadecane #	ERGOST-5-EN-3-OL
Sm-68	HEXADECANOIC ACID $$ HEXADECANOATE $$ PALMITATE $$ PALMITIC ACID	ERGOSTA-8,25-DIEN-3-ONE, 14,24-DIMETHYL-
Sm-69	Hexadecanoic acid, 1,5-pentanediyl ester $$ Palmitic acid, pentamethylene ester $$ 1,5-Pentanediol dipalmitate $$ 5-(Palmitoyloxy)pentyl palmitate #	ETHANOL, 2,2'-DITHIOBIS-
Sm-70	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester $$ Palmitin, 2-mono- $$ Palmitic acid .beta.-monoglyceride $$ 2-Hexadecanoyl glycerol	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE
Sm-71	HEXADECANOIC ACID, METHYL ESTER $$ METHYL HEXADECANOATE $$ PALMITIC ACID METHYL ESTER $$ EMERY 2216	Guanosine $$ Guanine, 9.beta.-d-ribofuranosyl-
Sm-72	ICOSANE $$ EICOSANE $$ EICOSAN $$ N-EICOSANE	Heptacosane, 1-chloro-
Sm-73	n-Hexadecanoic acid $$ Hexadecanoic acid $$ n-Hexadecoic acid $$ Palmitic acid	HEXADECANOIC ACID
Sm-74	Nickel(II) bis(N,N-dihexyldithiocarbamate)	Hexadecanoic acid, ethyl ester
Sm-75	NONACOSANOL	Imidazole-4-carboxylic acid, 2-fluoro-1-methoxymethyl-, ethyl ester
Sm-76	NONADECANE $$ N-NONADECANE	IRON, TRICARBONYL[(2,3,4,5-.ETA.)-2,3,4,5-TETRAHYDROXY-2,4-CYCLOPENTADIEN-1-ONE]-
Sm-77	Octacosane $$ n-Octacosane	l-Gala-l-ido-octose
Sm-78	OCTADECANOIC ACID $$ STEARATE $$ STEARIC ACID $$ 1-HEPTADECANECARBOXYLIC ACID	Linoleic acid ethyl ester
Sm-79	Octadecyl trifluoroacetate $$ Octadecyl 2,2,2-trifluoroacetate $$ 1-Octadecanol, trifluoroacetate $$ Trifluoroacetic acid, n-octadecyl ester	Methyl 2-methylsulfonyl-.alpha.-d-xylofuranoside
Sm-80	Palmitic acid vinyl ester $$ Hexadecanoic acid, ethenyl ester $$ Vinyl palmitate #	N-Acetyl-d-serine
Sm-81	PENTACOSANE $$ N-PENTACOSANE	n-Hexadecanoic acid
Sm-82	PIPERIDINE, 1-[1,4-DIOXO-5-(1-PIPERIDINYL)PENTYL]- $$ 1,5-DI-N-PIPERIDYL-1,4-DIOXO-PENTANE $$ 5-(N-PIPERIDYL)-4-OXOPENTANOIC ACID PIPERIDIDE	Oleic Acid
Sm-83	Squalene $$ 2,6,10,14,18,22-Tetracosahexaene, 2,6,01,15,19,23-hexamethyl-$$ Skvalen $$ Spinacene	Oleyl alcohol, heptafluorobutyrate
Sm-84	STIGMAST-5-EN-3-OL $$ STIGMAST-5-EN-3-OL, (3.BETA.,24S)- $$ (3BETA,24S)-STIGMAST-5-EN-3-OL $$ .BETA.-DIHYDROFUCOSTEROL	PENTADEUTERIO-2-ACETYL-1-PYRROLINE
Sm-85	STIGMASTA-5,22-DIEN-3-OL $$ STIGMASTA-5,22E-DIEN-3B-OL	Pentanoic acid, 4-methyl-, ethyl ester
Sm-86	Tetradecanoic acid $$ Myristic acid $$ n-Tetradecanoic acid $$ n-Tetradecoic acid	Propanamide, N,N-dimethyl
Sm-87	Triacontyl acetate $$ n-Triacontyl acetate $$ 1-Triacontanol, acetate	PROPENE-3,3,3-D3
Sm-88	TRICOSANE $$ N-TRICOSANE	Propenoic acid, 2-trifluoroacetylamino-

Sm-89	Tricosyl trifluoroacetate	STIGMAST-5-EN-3-OL
Sm-90	Vitamin E $$ 2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]- $$ .alpha.-Tocopherol $$ .alpha.-Tokoferol	Stigmasterol
Sm-91	Z,E-2,13-Octadecadien-1-ol	Tetraacetyl-d-xylonic nitrile
Sm-92	Z-12-Pentacosene	TETRADECANOIC ACID, ETHYL ESTER
Sm-93	Z-14-Nonacosane	Z-5-Methyl-6-heneicosen-11-one