CBRNE Def. Stud. 1(1), June 2026, 7-12

CBRNE Defense Studies

Journal homepage: https://journal.solusiriset.com/index.php/cds

Original Article

Characterization of bioactive compounds in NPK biofertilizer as a sustainable plant growth promoter to mitigate CBRN environmental and chemical risks

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https://doi.org/10.55749/cds.v1i1.167
Received: 10 May 2026; Revised: 24 May 2026; Accepted: 11 Jun 2026; Published online: 20 Jun 2026; Published regularly: 30 Jun 2026

Abstract— Metabolite analysis of NPK fertilizers based on biofertilizers was performed using Gas Chromatography-Mass Spectrometry (GC-MS) technique to identify the presence of bioactive compounds inside fertilizers. The identification of the metabolites revealed the presence of various metabolite compounds such as fatty acids, esters, alcohols, and sterol derivatives. Identified compounds were mapped using MetaboAnlayst and showed the association with steroid biosynthesis pathway. This pathway is associated with plant sterol, ergosterol, and brassinosteroid biosynthesis, which play roles in plant growth and soil microorganisms' activity. The existence of compounds like oleic acid and sterol derivatives indicates that NPK fertilizer can be utilized not only as a source of macronutrients but also as a biofertilizer that enhances soil biological activities and plant growth.

Keywords—*Biofertilizer; CBRN mitigation; Metabolites; NPK fertilizer; Steroid biosynthesis*

1. INTRODUCTION

NPK fertilizer is one of the most commonly used fertilizers for supplying essential macronutrients, namely nitrogen (N), phosphorus (P), and potassium (K), required for plant growth [1]. In addition to its macronutrient composition, advances in modern fertilizer technology have shown that fertilizers may also contain bioactive compounds and beneficial microorganisms functioning as biofertilizers [2,3].

Bioactive compounds and biofertilizers have the ability to improve soil fertility through the biochemical activities of microorganisms and the production of secondary metabolites. These metabolites may include fatty acids, sterols, terpenoids, and other organic compounds that contribute to plant growth and support soil microbial activity [4].

Beyond their conventional role in boosting agricultural yields, these microbial metabolites and bioactive constituents hold a critical, yet underdeveloped, potential in environmental security. In the context of contemporary ecological threats, agroecosystems are increasingly vulnerable to anthropogenic degradation, including contamination from hazardous chemical agents and industrial pollutants [5]. The integration of functional biofertilizers offers a dual-action mechanism; while macronutrients restore the baseline fertility of depleted soils, the inherent bioactive compounds stimulate adaptive plant physiological responses and fortify the soil microbiome against chemical stressors. Consequently, leveraging these biological features presents a strategic paradigm shift toward sustainable risk management. By characterizing these specific secondary metabolites, this study aims to evaluate their efficacy not only as primary growth promoters but also as natural biostimulants capable of mitigating chemical, biological, radiological, and nuclear (CBRN) environmental and chemical risks in vulnerable soils.

Metabolite analysis using Gas Chromatography–Mass Spectrometry (GC-MS) is one of the widely applied techniques for the characterization of bioactive compounds in fertilizers compared to genetic analysis, i.e., gene expression or gene editing [6-7]. Besides compound identification, a metabolomics approach can also be performed through metabolic pathway analysis using the MetaboAnalyst platform and related metabolite databases [8-9].

2. EXPERIMENTAL SECTION

2.1. Materials

Metabolite extraction and GC-MS analysis in this study were conducted following the metabolomics procedure described by Fendiyanto et al. (2020) [10]. The NPK biofertilizer sample was prepared through solvent extraction using absolute alcohol and incubated overnight to optimize metabolite dissolution prior to analysis. The extracted solution was subsequently filtered and subjected to Gas Chromatography–Mass Spectrometry (GC-MS) analysis for metabolite profiling. Compound

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identification was performed based on retention time and mass spectral fragmentation patterns using available metabolite databases. Furthermore, the detected metabolites were analyzed using MetaboAnalyst for metabolomic interpretation and pathway enrichment analysis. This approach enabled the identification of several bioactive compounds, including fatty acids, sterols, terpenoids, and other secondary metabolites associated with biological activity in biofertilizer samples, consistent with the metabolomic analysis approach reported by Fendiyanto et al. (2020) [10].

The material used in this study was NPK biofertilizer fertilizer sample. Absolute alcohol was used as the extraction solvent during sample preparation prior to metabolite analysis.

2.2 Instrumentation

Metabolite analysis was performed using Gas Chromatography–Mass Spectrometry (GC-MS) for the identification of bioactive compounds present in the NPK biofertilizer sample. The obtained metabolite data were further analyzed using MetaboAnalyst and several metabolite databases integrated within the platform.

2.3 Sample Preparations

Approximately 10 ml of NPK biofertilizer sample was immersed in absolute alcohol and incubated overnight to allow metabolite extraction. After incubation, the extract solution was filtered to remove solid particles and prepared for GC-MS analysis.

2.4 GC-MS Analysis

The prepared extract was injected into the GC-MS instrument for metabolite profiling. Compounds were identified based on retention time and mass spectral fragmentation patterns. The detected metabolites included several bioactive compounds such as fatty acids, sterol derivatives, alcohols, and other organic compounds.

2.5 Metabolite and Pathway Analysis

The metabolite data obtained from GC-MS analysis were processed using MetaboAnalyst software followed Fendiyanto et al. (2024) [11]. Compound annotation and pathway mapping were performed using databases available within MetaboAnalyst, including the Kyoto Encyclopedia of Genes and Genomes (KEGG). The identified metabolites were associated with several metabolic pathways, particularly steroid biosynthesis pathways related to bioactive compound production.

3. RESULT AND DISCUSSION

3.1 Metabolite Profiling of NPK Biofertilizer Using GC-MS

The metabolite characterization of the NPK biofertilizer was successfully carried out using Gas Chromatography–Mass Spectrometry (GC-MS) (**Table 1**). The chromatogram analysis revealed the presence of several bioactive compounds belonging to different metabolite groups, including fatty acids, sterol derivatives, alcohols, esters, and terpenoid-related compounds [12]. The detected metabolites were further annotated using metabolite databases integrated within MetaboAnalystR. A similar metabolomics-based GC-MS approach has previously been reported by Miftahul Huda Fendiyanto et al. (2020) [10], demonstrating that metabolite profiling is effective for identifying biologically active compounds associated with lipid and secondary metabolite pathways.

The identified compounds indicate that the NPK fertilizer does not only act as a source of essential nutrients but also contains biologically active metabolites that may contribute to improving soil fertility and supporting plant growth. Previous studies have reported that biofertilizers containing microbial metabolites and secondary compounds can enhance nutrient availability and stimulate microbial activity in the rhizosphere [13].

Among the detected metabolites, oleic acid was identified as one of the major fatty acid compounds present in the sample. Oleic acid is known to play important roles in membrane stability, microbial metabolism, and plant responses to environmental stress. Fatty acids and lipid-derived metabolites are commonly associated with membrane biosynthesis and environmental adaptation mechanisms in microorganisms [11]. In addition, sterol-related metabolites such as Estriol-17-glucuronide suggest the presence of steroid-associated metabolic activity that may contribute to biological signaling and interactions within soil ecosystems.

3.2 Metabolic Pathway Analysis Using MetaboAnalystR

The metabolite data obtained from GC-MS analysis were further processed using MetaboAnalystR for pathway enrichment analysis. MetaboAnalystR has been widely applied in metabolomics studies for compound annotation, pathway analysis, and biological interpretation of metabolite datasets [8-9].

The analysis showed that several identified metabolites were associated with steroid biosynthesis and lipid metabolism pathways (**Figure 1**). The pathway mapping demonstrated the involvement of sterol-related compounds in biochemical processes connected to terpenoid and steroid metabolism. Steroid biosynthesis pathways are particularly important because they are linked to the production of phytosterols, ergosterols, and other lipid-derived metabolites involved in microbial activity and plant physiological regulation.

The presence of metabolites associated with lipid metabolism suggests that the NPK biofertilizer may provide additional biological benefits beyond its conventional role as a macronutrient fertilizer. Lipid-derived metabolites are known to contribute to microbial membrane formation, nutrient transport, and stress adaptation mechanisms in soil microorganisms [6-7,11]

The pathway analysis generated using MetaboAnalystR demonstrated that several metabolites detected in the NPK biofertilizer were associated with steroid biosynthesis and lipid-related metabolic pathways. The green-highlighted components in the

Table 1. Selected metabolites identified in NPK biofertilizer using GC-MS and MetaboAnalystR

No	Query	Match	HMDB	PubChem	KEGG
1	ethyl denoecta hydro	3,4-Dimethylideneoctadecanylcarnitine	HMDB0240793	NA	NA
2	Cyclo hexane methanol	Methanol	HMDB0034717	10353	NA
3	Hexadecanoic acid	Methyl dodecanoate	HMDB0031018	8139	NA
4	Cyclic octatomic sulfur	NA	NA	NA	NA
5	Trimethyl bicyclo (3.1.1) heptan epoxy-6-one	NA	NA	NA	NA
6	Octadecenoic acid	Oleic acid	HMDB0000207	445639	C00712
7	Octadienoic acid	4-isopropylidene-6	7-dimethyl methyl ester	NA	NA
8	Bromoadic acid	octadecyl ester	NA	NA	NA
9	Estra-1 3 5 (10)-trien-17.beta.-ol	Estriol-17-glucuronide	HMDB0010333	151453	C11346
10	Heptadecane	Heptadecane	HMDB0059830	12398	C01816
11	Trans-2-Methoxycinnamic acid	heptyl ester	NA	NA	NA
12	Tetracosane	Lignocerane	HMDB0034282	12952	NA
13	Pentacosane	Isopentacosane	HMDB0031069	527459	NA
14	Bis (2-ethylhexyl) phthalate	Bis(2-ethylhexyl) phthalate	HMDB0249243	NA	C03690
15	Eicosane	Eicosane	HMDB0059909	8222	NA
16	Octacosane	Octacosane	HMDB0061868	12408	NA
17	13-Docosenamide	NA	NA	NA	NA
18	Squalene	Squalene	HMDB0000256	638072	C00751
19	Cholesta	7-Dehydrocholesterol	HMDB0000032	439423	C01164
20	Cholesterol	Cholesterol	HMDB0000067	5997	C00187
21	Benzene 1-tert-butyl-4-cyclopropyl methyl	NA	NA	NA	NA
22	Tri (2-ethylhexyl) trimellitate	NA	NA	NA	NA

pathway map represents enzymatic reactions linked to annotated metabolites identified from the GC-MS analysis. These metabolic reactions are involved in the conversion of precursor compounds into biologically important sterol derivatives such as phytosterols and steroid-associated metabolites.

The pathway map indicates that lipid-derived metabolites identified in the biofertilizer may participate in sterol formation through intermediate steroid biosynthesis reactions. Steroid biosynthesis is an important metabolic pathway because it produces sterol compounds that play major roles in membrane structure, signal transduction, and physiological regulation in plants and microorganisms. Several detected metabolites were associated with reactions involved in the formation of sterol intermediates related to compounds such as campesterol, sitosterol, and stigmasterol, which are commonly known as plant sterols or phytosterols.

Phytosterols are essential components in maintaining membrane stability and permeability in plant cells. In addition, sterol-derived metabolites are closely associated with the biosynthesis of brassinosteroids, which are plant growth-regulating hormones involved in cell elongation, vascular differentiation, seed germination, and stress tolerance. The presence of steroid-associated metabolic pathways in the NPK biofertilizer suggests that the fertilizer may contribute not only to nutrient supplementation but also to biological stimulation of plant growth. Furthermore, lipid metabolism identified in this pathway may also indicate active microbial metabolic processes occurring during biofertilizer production. Fatty acid and sterol-related metabolites are commonly synthesized by beneficial soil microorganisms to support membrane formation, environmental adaptation, and microbial survival under stress conditions. These microbial metabolites may indirectly enhance soil biological activity and improve nutrient availability in the rhizosphere.

The identification of steroid biosynthesis-related pathways through metabolomic analysis therefore indicates that the NPK biofertilizer contains functional bioactive metabolites with potential roles in promoting plant growth, improving stress resistance, supporting microbial activity, and enhancing overall soil fertility. Similar observations regarding lipid and sterol metabolism in metabolomics studies have also been reported by Miftahul Huda Fendiyanto et al. (2020) [10], who demonstrated that fatty acid and secondary metabolite pathways are closely associated with biological adaptation and metabolic regulation in plant-related systems.

3.3 Biological Significance of Detected Metabolites

The identified metabolites indicate the presence of bioactive compounds that may support soil microbial activity and plant development. Previous studies have shown that biofertilizers enriched with microbial metabolites can improve soil biochemical properties and enhance nutrient cycling efficiency [2].

Fatty acid compounds such as oleic acid may help maintain microbial cell membrane integrity and improve nutrient absorption efficiency within the rhizosphere. Fatty acid metabolites are also associated with environmental stress tolerance and cellular signaling processes in both plants and microorganisms [10].

The detection of steroid- and sterol-related metabolites in this study may also indicate microbial fermentation activity during biofertilizer production. Sterol compounds are commonly associated with membrane stabilization and signaling processes in microorganisms and plants. Furthermore, terpenoid-associated pathways identified through MetaboAnalystR suggest potential roles in plant defense responses and adaptation to environmental

stress [14]. These findings support the hypothesis that NPK biofertilizer contains functional metabolites that may improve soil biological quality in addition to supplying essential macronutrients.

3.4 Relationship Between Metabolite Composition and Biofertilizer Function

The metabolomic profile obtained in this study demonstrates that the NPK biofertilizer possesses a complex biochemical composition. The presence of fatty acids, sterol derivatives, alcohols, and ester compounds suggests that the fertilizer may function not only as a nutrient source but also as a biologically active soil amendment.The integration of GC-MS-based metabolite profiling with MetaboAnalystR pathway analysis provided more comprehensive information regarding the biochemical characteristics of the fertilizer. This metabolomics approach allows a deeper understanding of bioactive compound composition and their potential biological roles in sustainable agricultural applications, consistent with previous metabolomics studies reported by Pang et al. (2022) [8] and Fendiyanto et al. (2020) [10].

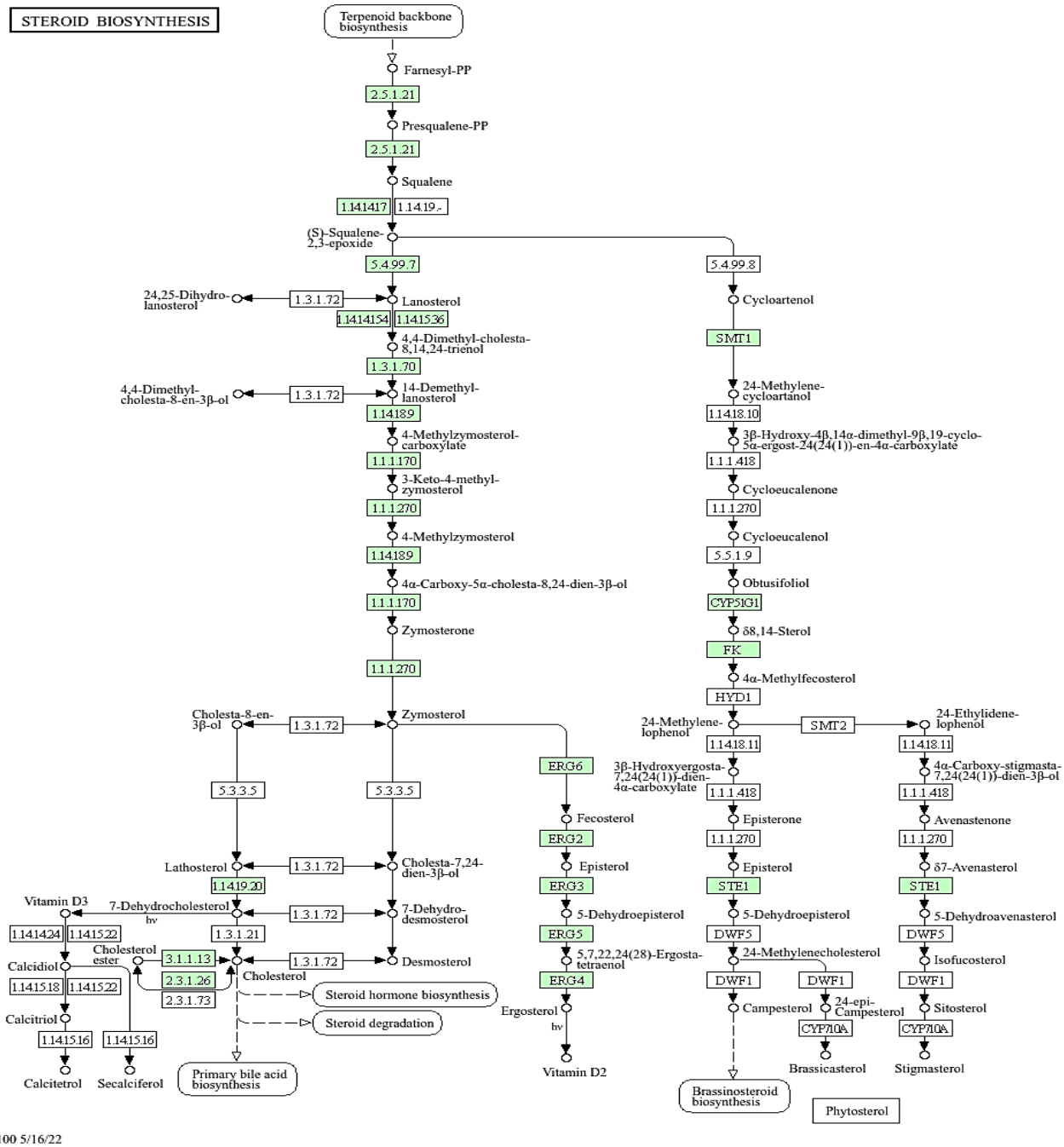

Figure 1. Steroid biosynthesis pathway generated from MetaboAnalystR analysis of NPK biofertilizer metabolites

4. CONCLUSION

In conclusion, metabolomic interpretation using MetaboAnalystR successfully identified key bioactive compounds within the NPK biofertilizer, notably those associated with steroid biosynthesis and lipid metabolism pathways. These findings indicate that the NPK biofertilizer extends beyond its traditional role as a macronutrient source; it functions as a potent biostimulant that enhances beneficial microbial activity, restores soil fertility, and promotes resilient plant growth. Crucially, from a CBRN risk mitigation perspective, these specific lipid and steroid metabolites play a vital role in fortifying the soil-plant system against external stressors. By stabilizing microbial cell membranes and inducing systemic tolerance in plants, these compounds provide a biological buffer capable of mitigating environmental toxicity and chemical hazards. Overall, this study demonstrates that metabolomics-based approaches offer a highly effective framework for characterizing biofertilizers, establishing them as sustainable, dual-action agents for both quality evaluation in agriculture and environmental risk management in CBRN-vulnerable ecosystems.

5. AUTHOR'S DECLARATION

5.1. Supporting Information

Supporting information for this study includes additional GC-MS chromatograms, metabolite identification tables, annotated metabolite datasets processed using MetaboAnalystR, and pathway analysis figures related to steroid biosynthesis and lipid metabolism identified in the NPK biofertilizer sample.

5.2. Acknowledgements

Authors acknowledge to Dr. Miftahul Huda Fendiyanto, S.Si., M.Si for GCMS analysis. The authors would like to express their sincere gratitude to the laboratory staff and academic supervisors who provided technical assistance and valuable guidance throughout this study. Appreciation is also extended to the institution and research facilities that supported the GC-MS analysis and metabolomics data processing using MetaboAnalystR. This research was conducted as part of academic research activities related to the characterization of bioactive compounds in biofertilizer products.

5.3. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

5.4. Author Contributions

M.A.Z, N.N, and M.P.P. conducted sample preparation, GC-MS analysis, metabolite annotation, data interpretation, and manuscript writing. All authors contributed to manuscript revision and approved the final version of the manuscript.

5.5. AI Statement

Gemini AI was performed to illustrate the graphical abstract. Chat GPT was utilize to enhance the clarity, grammar, and overall readability of this manuscript. All technical content, data interpretation, and conclusion were solely developed and verified by the authors. The final version of the manuscript was thoroughly reviewed to ensure accuracy, coherence, and alignment with the study's findings.

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