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RESEARCH ARTICLE

# Effectiveness of treated sludge as plant growth modulator on the phytometabolics of African marigold (*Tagetes erecta*)

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## Abstract

The current study investigated the effects of treated sludge on the phytochemical composition of African Marigold (*Tagetes erecta*) flowers. African Marigold flowers were cultivated with treated sludge and their phytochemical profiles were analyzed using gas chromatography-mass spectrometry (GC-MS). Flowers from plants treated with sludge increased in various bioactive compounds, including α-Pinene, linalool, β-Caryophyllene, quercetin and luteolin, recognized for their antioxidant, anti-inflammatory and antimicrobial properties. The study underscores the potential of treated sewage sludge as a sustainable soil amendment to improve plant growth and Marigold flowers' phytochemical profile. This study provides valuable prescience into agricultural practices that ensure sustainability and highlights the potential of sewage sludge as a beneficial resource for enhancing crop quality and bioactive compound content.

## Keywords

Treated Sludge; African marigold; phytometabolites; carotenoids

## Introduction

Marigold (*Tagetes erecta*), a member of the *Asteraceae* family, is well-known for its decorative qualities and considerable therapeutic potential (1). Its essential oil has been extensively researched and employed in various sectors, such as nematicide, cosmetics, the food industry and pest management (2-5). The essential oil extracted from *Tagetes* species exhibits multiple biological properties, such as "antimicrobial, antiparasitic, antiseptic and antispasmodic effects" (6). Traditional medicinal practices involving *Tagetes erecta* and *T. patula* include their use in decoctions as remedies for malaria and fever (7). "While much of the literature concerning the medicinal attributes of marigolds focuses on the wild species *Tagetes minuta,* preliminary phytochemical investigations of *Tagetes erecta* have demonstrated notable concentrations of alkaloids, phenolic compounds, flavonoids, salicylic acid and terpenes" (8).

African marigold (*Tagetes erecta L.*) is highly valued for its widespread cultivation as an ornamental plant and easy availability as loose flowers. "*Tagetes* species, especially *Tagetes erecta*, is recognized for

rich pigments like xanthophyll and bioactive compounds, offering potential applications in food and pharmaceutical industries. Exploring post-harvest techniques like oil extraction from flowers and other plant parts could significantly enhance the crop's worth. Moreover, this research may inform waste management strategies for discarded flowers and leaves used in various decorations. However, there's a noticeable lack of information on oil extraction and phytochemical composition of African marigolds" (*Tagetes erecta L.*) (9).

Sewage sludge (SS), a byproduct of wastewater treatment, is generated significantly within urban areas (10). Globally, it is estimated that between 75 to 100 million tons of SS is produced annually, with projections suggesting a rise to 127.5 million tons by 2030 (11). "Due to its complex composition containing both organic and inorganic chemicals, SS has the potential to contaminate soil and water ecosystems. Consequently, the management of SS has become a critical environmental concern, especially in developing countries. Improper handling and disposal of SS can lead to various environmental problems, including soil and water pollution, emission of greenhouse gases and spread of diseases" (12). Addressing these issues requires effective sludge management strategies to protect public health, safeguard the environment and preserve precious water resources.

In recent years, there's been a rising trend in using sewage sludge (SS) to boost soil fertility in agriculture (13). Various methods are employed for SS management, including anaerobic digestion, composting, incineration and landfill disposal (14). Sewage sludge is dried and used as a soil amendment to enhance soil quality for agriculture (15). Composting is another standard method for SS management, stabilizing organic matter and nutrients for easier handling and use (16). However, incineration of SS, while effectively removing organic debris, can release harmful toxins like dioxins and furans into the atmosphere" (13). Similarly, though typical, landfill disposal of SS poses risks of groundwater contamination, as recent studies suggest (17).

Employing pre-digested sewage sludge (SS) in agriculture is widely recognized as a sustainable approach to enhance crop yield and soil quality (18). "Due to its nutrient-rich composition, SS serves as an effective fertilizer for crops such as fruits, vegetables and grains (19), contributing to soil fertility and structure by supplying essential macronutrients like nitrogen (N), phosphorus (P) and potassium (K), as well as micronutrients including zinc (Zn), iron (Fe) and copper (Cu) (20). Various non-edible crops, such as marigold for flowers, jatropha for energy, bamboo for construction, *Sesbania* spp. for fibre, borage for pharmaceuticals and rubber for biopolymers, can be cultivated utilizing controlled SS doses" (21).

Marigold flowers, in particular, dominate the market with an annual production of 1754 thousand metric tons, highlighting their significant commercial value (22). Given that marigold (*Tagetes erecta L.*) flowers are predominantly used for non-edible purposes, the risk of human exposure to heavy metals and contaminants through consumption is minimized (23). Consequently, marigold cultivation emerges as a promising solution for managing sewage sludge (SS), offering a viable and sustainable approach to repurposing this material.

The following objectives were addressed during the study. To determine the Phyto metabolite composition of flowers grown on treated sludge using gas chromatography-mass spectrometry (GC-MS) and to separate and identify various secondary metabolites in Marigold flowers grown on treated sludge.

## Materials and Methods

### Experimental site and geography

This study was conducted at Floriculture Research Station, Tamil Nadu Agricultural University, Thovalai, Kanyakumari Dt., Tamil Nadu, India, at a longitude of 8.2312° N and latitude of 77.5060° E and an elevation of 81 meters above mean sea level.

### Field Preparation and planting

The experimental field was ploughed thoroughly to bring the soil to a fine tilth. After uniform levelling, the field was prepared with ridges and furrows with a plot size of 3x2 m. The seeds were sown in protrays at one seed per cell. The seeds germinated within a week. Thirty-day-old healthy seedlings were used for planting. The seedlings were planted at a spacing of 45x35 cm in the field. Gap filling was done on the 7th day after transplanting with seedlings obtained from the same sowing date.

### Treatment with treated sludge

The treated sludge was incorporated into the soil as a basal dose per the treatment specifications to evaluate its performance enhancement plant growth in African Marigold (*Tagetes erecta*). This experiment was laid out in a Randomised Block Design, and the total number of treatments was listed. T1 -Absolute control (FYM 25t/ha), T2 - FYM 25t/ha + Recommended Dose of Fertilizer (RDF) 90:90:75 kg NPK/ha, T3 - Treated sludge -2.5 t/ha, T4 - Treated sludge - 5.0 t/ha, T5 - RDF (90:90:75 kg NPK/ha) + Treated sludge 2.5t/ha, T6 -RDF (90:90:75 kg NPK/ha) +Treated sludge 5 t/ha, T7 - RDF (90:90:75 kg NPK/ha) + Treated sludge 2.5t/ha + Micronutrients (FeSO4 0.5% + ZnSO4 0.5%), T8 - RDF (90:90:75 kg NPK/ha) + Treated sludge 5.0t/ha + Micronutrients (FeSO4 0.5% + ZnSO4 0.5%) and T9 - RDF (90:90:75 kg NPK/ha) + Micronutrients (FeSO4 0.5% + ZnSO4 0.5%). The treatment T7 - RDF (90:90:75 kg NPK/ha) + Treated sludge 2.5t/ha + Micronutrients (FeSO4 0.5% + ZnSO4 0.5%) was selected due to its best performance in both morphological and floral characteristics and was subjected to GCMS analysis. This was compared with control sample T1 -Absolute control (FYM 25t/ha).

The sludge gathered from wastewater treatment facilities underwent composting to eradicate pathogens and enrich its nutrient content. The treated sludge was then incorporated as a soil supplement for the African

Marigold plants. This application was performed once during the transplanting stage of plant development.

### Collection of Flower Samples:

Fully developed Marigold flowers treated with sludge at 2.5 t/ha were gathered from the plants during their peak blooming period 60 Days after Transplanting. The harvesting procedure was conducted meticulously to ensure minimal damage or contamination to the flowers (9)

### Phyto Metabolite Extraction:

The phytochemicals in the harvested Marigold flower samples were extracted using a solvent extraction method, targeting compounds such as flavonoids, carotenoids, triterpenoids, phenolic acids, alkaloids and essential oils. Initially, the flowers were finely ground into a powder using liquid nitrogen, after which they were subjected to extraction using a suitable solvent - methanol. A Soxhlet extractor was utilized during the extraction process to enhance extraction efficiency (9).

### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis:

### Sample Preparation for GC-MS Analysis:

The phyto metabolites, after isolation, were subjected to concentration and derivatization techniques to improve their volatility and stability, preparing them for GC-MS analysis. The mixture was subjected to sonication for 30 minutes at room temperature. It was then centrifuged at 4000 rpm for 10 minutes and the supernatant was collected (24). The supernatant was transferred to a round bottom flask. A rotary evaporator was used to evaporate the solvent under reduced pressure. The water bath temperature was set to 40°C and gradually, the pressure was reduced to 100 mbar (25). 50 µL of the concentrated extract was added to a 2 mL vial for esterification. 100 µL of 14% boron trifluoride (BF3) was added in methanol. The vial was capped and vortexed for 30 seconds. It was heated at 80°C for 20 minutes. The derivatized sample was allowed to cool to room temperature. 300 µL of hexane and 300 µL of water were added to the methylated samples and vortexed for 1 minute and the upper hexane layer was collected (26). The sample was filtered through a 0.22 µm Poly Tetra Fluoro Ethylene syringe filter. 200 µL of the filtered, derivatized sample was transferred to a clean GC vial with a 250 µL glass insert. The vial was capped securely (27). Then, the prepared samples were loaded into the GC-MS autosampler.

### Instrumentation and Chromatographic Conditions:

Gas chromatography-mass spectrometry (GC-MS) analysis was performed using a gas chromatograph coupled to a mass spectrometer. The standard experiment was conducted utilizing Agilent GC 7890A/MS5975C using a Capillary Column after the extract had been dissolved in methanol before analysis. With an injector operating in split mode with helium, the sample was introduced into the Agilent DB5MS apparatus, which has a column length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 microns. The retention time and fragmentation pattern were assessed using the National Institute of Standards and Technology particular library to identify the extract's bioactive ingredients. Chromatographic separation was achieved with a capillary column selected explicitly for the compounds in the African marigold being analyzed. Parameters such as temperature programming and flow rates were carefully adjusted to achieve the best possible separation and detection of phyto metabolites (28). The Mass Spectrometric Conditions are as follows: Ionization Source - Electron Impact (EI) ionization at 70 eV, Ion Source Temperature - Set to 230°C, Transfer Line Temperature - Set to 280°C, Mass Analyzer - quadrupole mass analyzer, Acquisition Mode - Full scan mode, mass range: m/z 50-550, Scan Rate - 3.5 scans/sec (29,30).

### Identification of Phyto Metabolites:

The phyto metabolites in marigold flower samples were identified by comparing the retention times and mass spectra of detected peaks with those of authentic standards and databases.

### Quantification of Phyto Metabolites:

The process of quantifying identified phyto metabolites in the Marigold flower samples included using external calibration curves created with known concentrations of standard compounds. Peak areas acquired from GC-MS analysis were integrated and concentrations were established by referencing the calibration curves.

### Quality Control Measures:

During the GC-MS analysis, various quality control measures, as listed below, were implemented to ensure the reliability and validity of the results. These measures included utilizing blanks, standards and replicate analyses to monitor instrument performance, detect contaminants and assess method accuracy and precision. Sigma-Aldrich (St. Louis, MO) provided the DPPH (2,2-diphenyl-1-picrylhydrazyl). Methanol and the standard Ascorbic Acid were procured from Sai Scientifics, India.

## Results and Discussion

The analysis of Marigold flower samples using GC-MS revealed a diverse range of phyto-metabolites, each contributing to the unique chemical composition of the flowers (Fig. 1. & 2). Examination of the chromatograms generated from the analysis exhibited multiple peaks corresponding to various compounds present in the samples. By meticulously interpreting these chromatograms and comparing them with the spectral library (NIST), several key phyto metabolites were successfully identified and characterized. Among the compounds identified, flavonoids emerged as prominent constituents of Marigold flowers. Specifically, flavonoids quercetin, kaempferol and luteolin derivatives were detected, which are responsible for the vibrant shades of orange colours seen in Marigold petals. In addition to their visual appeal, these flavonoids possess antioxidant properties, offering potential health benefits to consumers.

Furthermore, carotenoids were significant phyto metabolites in Marigold flowers, responsible for the characteristic yellow to red colours observed in various plant tissues, including petals. Their presence, particularly lycopene and β-carotene, enhances the visual appeal of

Marigold flowers and may offer health benefits due to their antioxidant properties and provitamin A potential. Moreover, terpenoids were abundant in the phytochemical profile of Marigold flowers, representing a diverse group of compounds with numerous biological activities, including antimicrobial and anti-inflammatory properties. Identifying terpenoids such as limonene and β-caryophyllene highlights the medicinal potential of Marigold flowers and underscores their significance in traditional medicine and herbal remedies.

Alkaloids, another category of phyto metabolites, were detected in Marigold flower samples, albeit in smaller quantities than flavonoids, carotenoids and terpenoids. Alkaloids play diverse roles in plants, including defence against herbivores and pathogens. The discovery of alkaloids like santonin in Marigold flowers contributes to the plant's chemical complexity and may impact its ecological interactions and pharmacological properties. Furthermore, phenolic compounds were identified as essential components of Marigold flowers. Phenolic compounds exhibit antioxidant and anti-inflammatory properties, making them beneficial for human health. The presence of phenolic compounds such as chlorogenic acid and caffeic acid derivatives underscores the potential health advantages associated with the consumption or medicinal use of Marigold.

Employing treated sludge as a soil amendment enhanced plant growth and crop productivity. This investigation delves into the influence of treated sludge on the phytochemical composition of African Marigold (*Tagetes erecta*) flowers through gas chromatography-mass spectrometry (GC-MS) analysis. The study seeks to elucidate the role of enriched phyto metabolites in improving the quality and bioactivity of Marigold flowers cultivated in soil amended with sludge. The results of the GC-MS analysis revealed significant differences in the phytochemical profiles of Marigold flowers grown on treated sludge compared to those cultivated under control conditions. Several essential compounds were identified and characterized, providing insights into the biochemical responses of Marigold plants to soil amendment with treated sludge, as depicted in Fig.1 below.

One notable discovery from the study is the heightened presence of α-Pinene, a monoterpene compound, in Marigold flowers derived from plants treated with sludge. α-Pinene is known for its antimicrobial and anti-inflammatory properties and contributes to the characteristic scent of Marigold flowers. The increased levels of α-Pinene suggest a potential adaptive response of Marigold plants to the nutrient-rich environment provided by treated sludge, leading to enhanced biosynthesis of this bioactive compound. The retention time for the α-Pinene peak was observed at 10.908 minutes with a peak area of 10.51 % for the flower sample grown by incorporating with treated sludge at 2.5 t/ha (Table 1) while for the control treatment, the retention time of α-Pinene peak was observed at 10.153 minutes with the peak area of 0.62 % (Table 2). Additionally, the analysis revealed elevated concentrations of linalool, β-Caryophyllene, quercetin and luteolin in Marigold flowers cultivated with treated sludge. Linalool, classified as a monoterpene alcohol, exhibits antioxidant and anti-inflammatory properties, while β-Caryophyllene, categorized as a sesquiterpene hydrocarbon, demonstrates anti-inflammatory and analgesic effects. Quercetin and luteolin, both flavonoid compounds, are well-known for their potent antioxidant and anti-inflammatory attributes. The increased abundance of these phytochemicals suggests a synergistic reaction of Marigold plants to the favourable soil conditions

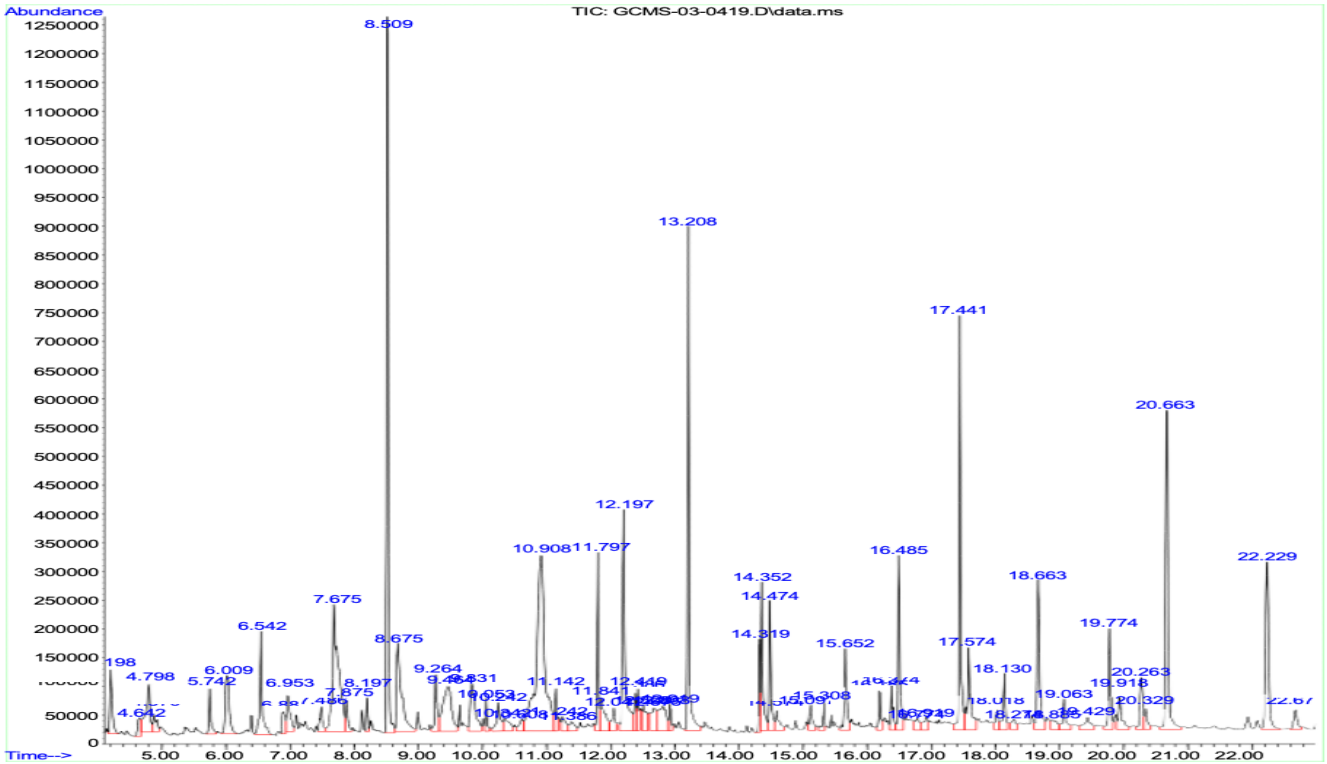


**Fig. 1.** Gas Chromatography-Mass Spectrometry (GC-MS) analysis highlighting the influence of treated sludge at 2.5t/ha on the phytochemical composition of African Marigold (*Tagetes erecta*)

**Table 1:** List of various phytochemicals identified by GC-MS analysis in the floral extract of African marigold (S1 - RDF (90:90:75 kg NPK/ha) + Treated sludge 2.5t/ha + Micronutrients (FeSO4 0.5% + ZnSO4 0.5%)

| Peak No. | Name of the compound | Retention time | Peak Area (%) | Molecular Formula | Molecular weight (g/mol) |
|---|---|---|---|---|---|
| 1 | Cyclohexanone | 4.198 | 1.11 | $C_6H_{10}O$ | 98.14 |
| 2 | 3,3-Dimethyl-2-butanol | 4.642 | 0.45 | $C_6H_{14}O$ | 102.17 |
| 3 | 1,3,4-Thiadiazol-2-amine | 4.798 | 1.38 | $C_2H_3N_3S$ | 101.13 |
| 4 | 1-Penten-3-ol | 4.876 | 0.40 | $C_5H_{10}O$ | 86.13 |
| 5 | Thymine | 5.742 | 0.63 | $C_5H_6N_2O_2$ | 126.11 |
| 6 | 1-Butanol | 6.009 | 1.64 | $C_4H_{10}O$ | 74.12 |
| 7 | Cinnoline | 6.542 | 1.59 | $C_8H_6N_2$ | 130.15 |
| 8 | Propenamide | 6.887 | 0.51 | $C_3H_5NO$ | 71.08 |
| 9 | 2(3H)-Furanone | 6.953 | 0.84 | $C_4H_4O_2$ | 84.07 |
| 10 | Silane | 7.486 | 0.50 | $SiH_4$ | 32.12 |
| 11 | 1,2-Benzenediol | 7.675 | 5.26 | $C_6H_6O_2$ | 110.11 |
| 12 | Benzene | 7.875 | 0.46 | $C_6H_6$ | 78.11 |
| 13 | 2-Methoxy-4-vinyl phenol | 8.197 | 0.34 | $C_9H_{10}O_2$ | 150.17 |
| 14 | 3-Amino-2,6-dimethoxy pyridine | 8.509 | 8.35 | $C_7H_{10}N_2O_2$ | 154.17 |
| 15 | Pyrazole-5-carboxylic acid | 8.675 | 2.89 | $C_4H_4N_2O_2$ | 112.09 |
| 16 | Allopurinol | 9.264 | 0.96 | $C_5H_4N_4O$ | 136.11 |
| 17 | Ethanamine | 9.464 | 2.72 | $C_2H_7N$ | 45.08 |
| 18 | D-Allose | 9.831 | 1.71 | $C_6H_{12}O_6$ | 180.16 |
| 19 | Benzaldehyde | 10.053 | 0.33 | $C_7H_6O$ | 106.12 |
| 20 | n-Hexadecanoic acid | 10.242 | 0.73 | $C_{16}H_{32}O_2$ | 256.42 |
| 21 | Phenanthrene | 10.342 | 20.52 | $C_{14}H_{10}$ | 178.23 |
| 22 | Methyl .beta.-d-galactopyranoside | 10.608 | 0.38 | $C_7H_{14}O_6$ | 194.18 |
| 23 | Alpha - pinene | 10.908 | 10.51 | $C_8H_{16}$ | 112.21 |
| 24 | 4-Nitrobenzoic acid | 11.142 | 0.59 | $C_7H_5NO_4$ | 167.12 |
| 25 | Guanosine | 11.242 | 0.42 | $C_{10}H_{13}N_5O_5$ | 283.24 |
| 26 | 2-Butenedioic acid | 11.386 | 0.35 | $C_4H_4O_4$ | 116.07 |
| 27 | Linalool | 11.797 | 1.65 | $C_{10}H_{18}O$ | 154.25 |
| 28 | 2,2-Diallylpyrrolidine | 11.841 | 0.99 | $C_{11}H_{19}N$ | 165.28 |
| 29 | Quinuclidine | 12.041 | 0.51 | $C_7H_{13}N$ | 111.19 |
| 30 | 1,2,3,4-Tetramethoxybenzene | 12.197 | 4.14 | $C_{10}H_{14}O_4$ | 198.22 |
| 31 | Oxirane | 12.386 | 0.49 | $C_2H_4O$ | 44.05 |
| 32 | Muco-Inositol | 12.419 | 0.58 | $C_6H_{12}O_6$ | 180.16 |
| 33 | Neo-Inositol | 12.508 | 0.68 | $C_6H_{12}O_6$ | 180.16 |
| 34 | l-Inositol | 12.675 | 0.86 | $C_6H_{12}O_6$ | 180.16 |
| 35 | Scyllo-Inositol | 12.808 | 1.32 | $C_6H_{12}O_6$ | 180.16 |
| 36 | 2-O-Mesyl arabinose | 12.919 | 0.33 | $C_6H_{11}O_6S$ | 211.21 |
| 37 | Pentadecanoic acid | 13.208 | 5.27 | $C_{15}H_{30}O_2$ | 242.40 |
| 38 | Beta Caryophyllene | 14.319 | 1.03 | $C_{15}H_{24}$ | 204.35 |
| 39 | 9,12,15-Octadecatrienal | 14.352 | 1.85 | $C_{18}H_{30}O$ | 262.43 |
| 40 | Tridecanoic acid | 14.474 | 1.50 | $C_{13}H_{26}O_2$ | 214.34 |
| 41 | 2-Pyridinamine, | 14.574 | 0.49 | $C_5H_6N_2$ | 94.11 |
| 42 | Phenylethyl Alcohol | 15.097 | 0.47 | $C_8H_{10}O$ | 122.16 |
| 43 | Eicosane | 15.308 | 0.36 | $C_{20}H_{42}$ | 282.55 |
| 44 | Benzene | 15.652 | 1.54 | $C_6H_6$ | 78.11 |
| 45 | Dihydroxymaleic acid | 16.185 | 0.57 | $C_4H_4O_6$ | 148.07 |
| 46 | Cyclotetradecane | 16.374 | 0.67 | $C_{14}H_{28}$ | 196.37 |
| 47 | Quercetin | 16.485 | 2.16 | $C_{15}H_{10}O_7$ | 302.24 |
| 48 | 1,3-Cyclopentadiene | 16.774 | 0.43 | $C_5H_6$ | 66.10 |
| 49 | 2-Ethylacridine | 16.919 | 0.40 | $C_{15}H_{13}N$ | 207.27 |
| 50 | Eicosyl heptafluorobutyrate | 17.441 | 4.62 | $C_{24}H_{41}F_7O_2$ | 506.57 |
| 51 | Luteolin | 17.574 | 1.74 | $C_{15}H_{10}O_6$ | 286.24 |
| 52 | n-Dodecyl thioglycolate | 18.018 | 0.36 | $C_{14}H_{28}O_2S$ | 260.44 |
| 53 | Cyclopropane carboxylic acid | 18.130 | 0.87 | $C_4H_6O_2$ | 86.09 |
| 54 | Cyclotrisiloxane | 18.274 | 0.33 | $C_6H_{18}O_3Si_3$ | 222.46 |
| 55 | 1,2-Benzisothiazol-3-amine | 18.663 | 2.85 | $C_7H_6N_2S$ | 150.20 |
| 56 | Cyclotrisiloxane | 18.885 | 0.43 | $C_6H_{18}O_3Si_3$ | 222.46 |
| 57 | Cyclohexanone | 19.063 | 0.69 | $C_6H_{10}O$ | 98.14 |
| 58 | Butanamide | 19.429 | 0.58 | $C_4H_9NO$ | 87.12 |
| 59 | Benzenepropanenitrile | 19.774 | 1.56 | $C_9H_9N$ | 131.17 |
| 60 | gamma.-Tocopherol | 19.918 | 0.81 | $C_{28}H_{48}O_2$ | 416.68 |
| 61 | Salsoline | 20.263 | 1.02 | $C_{11}H_{15}NO_2$ | 193.24 |
| 62 | Cyclohexane | 20.329 | 0.42 | $C_6H_{12}$ | 84.16 |
| 63 | alpha.-Tocopherol | 20.663 | 5.78 | $C_{29}H_{50}O_2$ | 430.71 |
| 64 | Stigmasterol | 22.229 | 3.60 | $C_{29}H_{48}O$ | 412.69 |
| 65 | 2-Benzothiazolamine | 22.673 | 0.51 | $C_7H_6N_2S$ | 150.20 |

facilitated by treated sludge, resulting in the accumulation of bioactive metabolites with potential health benefits. For the flowers grown under the application of treated sludge at 2.5t/ha, the retention times for linalool, β-Caryophyllene, quercetin and luteolin were observed at 11.797 min, 14.319 min, 16.485 min and 17.574 min, respectively (Fig. 1.). The peak area recorded for linalool, β-Caryophyllene, quercetin and luteolin were 1.65%, 1.03%, 2.16% and 1.74% respectively (Table 1). The other control treatment showed lower retention times, which are as follows: The retention times for linalool, β-Caryophyllene, quercetin and luteolin were observed at 11.037 min, 14.138 min, 16.255 min and 17.118 min, respectively (Fig. 2.). The peak area recorded for linalool, β-Caryophyllene, quercetin and luteolin were 0.32%, 1.85%, 2.85% and 0.51% respectively (Table 2).

Many studies have focused on the sustainable utilization of sewage sludge for cultivating Marigolds (*Tagetes erecta L.*). However, several unanswered questions remain that warrant further investigation. Specifically, we need to understand the precise mechanisms governing the modulation of phytochemical biosynthesis in response to treated sludge application. Additionally, conducting long-term studies to assess the persistence of phytochemical enrichment and the potential environmental impacts of sludge utilization is crucial for ensuring the sustainability of this agricultural practice.

In a study, broiler chicken litter-based organic fertilizers were applied to greenhouse-cultivated French marigolds, resulting in increased plant growth parameters, including root rate, indicating the positive effect of organic fertilizers on marigold growth in greenhouse conditions (31). However, another study reported a total chlorophyll content of 0.75 mg/g in marigold leaves (32). Discrepancies in findings may arise from variations in factors such as strain type, soil composition and environmental conditions. Organic fertilization can enhance various crops' chlorophyll content (33). Additionally, peroxidase activity in marigold leaves ranged from 85 to 90 U/min/g and the influence of cultivar variation on antioxidant activity in marigold leaves was observed in a study.

Moreover, catalase activity ranging from 11.5 to 12.5 U/min/g in several marigold cultivars was also observed (34). However, marigold flowers did not display chlorophyll content, catalase, or peroxidase activities in all treatments. It is widely recognized that chlorophyll content in flowers is uncommon in developed stages, with only minimal amounts typically present in early developmental stages (35).

The total flavonoid contents of 1.1 and 3.7 mg/g DW in the leaves and flowers of Mexican marigolds were documented (32). "The increase in ascorbic acid levels in marigold leaves enhances the regulation of the redox state of photosynthetic electron carriers, thereby improving the photosynthesis mechanism (36). Additionally, the impact of the growing substrate on phenolic compounds, ascorbic acid and flavonoids in marigold plants is also emphasized (37). Recent research has highlighted the role of lutein in determining the yellow colour intensity of marigold

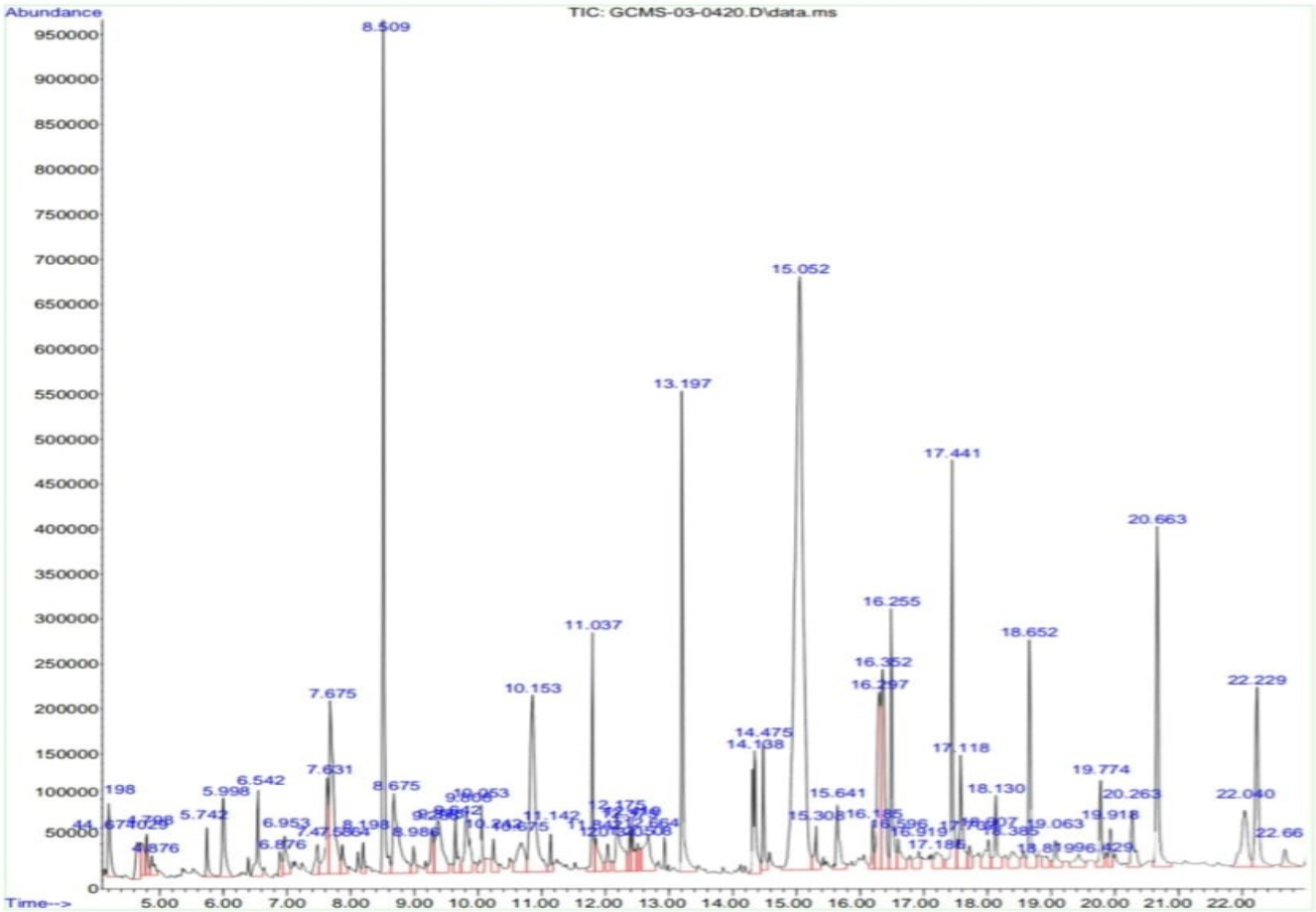


**Fig. 2.** Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the untreated plants (Control) on the phytochemical composition of African Marigold (*Tagetes erecta*)

**Table 2:** List of various phytochemicals identified by GC-MS analysis in the floral extract of African marigold (S2 - Absolute control: FYM 25t/ha)

| Peak No. | Name of the compound | Retention time | Peak Area (%) | Molecular Formula | Molecular weight |
|---|---|---|---|---|---|
| 1 | Cyclohexanone | 4.198 | 0.90 | $C_6H_{10}O$ | 98.14 |
| 2 | Benzonitrile | 4.642 | 0.81 | $C_7H_5N$ | 103.12 |
| 3 | Diglycerol | 4.709 | 0.56 | $C_6H_{14}O_5$ | 166.17 |
| 4 | Thiocyanic acid | 4.798 | 0.35 | HSCN | 59.08 |
| 5 | 4-Decene | 4.876 | 0.31 | $C_{10}H_{20}$ | 140.27 |
| 6 | Thymine | 5.742 | 0.41 | $C_5H_6N_2O_2$ | 126.11 |
| 7 | Acetic acid | 5.998 | 1.09 | $C_2H_4O_2$ | 60.05 |
| 8 | 2,5-Difluoroanisole | 6.542 | 0.88 | $C_7H_6F_{20}$ | 144.12 |
| 9 | Silane | 6.875 | 0.34 | $SiH_4$ | 32.12 |
| 10 | 1,2-Benzenediol | 6.953 | 0.57 | $C_6H_6O_2$ | 110.11 |
| 11 | Benzonitrile | 7.475 | 0.47 | $C_7H_5N$ | 103.12 |
| 12 | Propanoic acid | 7.631 | 1.33 | $C_3H_6O_2$ | 74.08 |
| 13 | 2-Methoxyresorcinol | 7.675 | 3.25 | $C_7H_8O_3$ | 140.14 |
| 14 | Octanoic acid | 7.864 | 0.38 | $C_8H_{16}O_2$ | 144.21 |
| 15 | Thymol | 8.198 | 0.28 | $C_{10}H_{14}O$ | 150.22 |
| 16 | Phenol | 8.509 | 6.24 | $C_6H_6O$ | 94.11 |
| 17 | 3-Methyl-2-furoic acid | 8.675 | 2.22 | $C_6H_6O_3$ | 126.11 |
| 18 | Piperazine | 8.986 | 0.32 | $C_4H_{10}N_2$ | 86.14 |
| 19 | 2-Hydroxy-3-methyl benzaldehyde | 9.286 | 0.69 | $C_8H_8O_2$ | 136.15 |
| 20 | Thiocyanic acid | 9.375 | 1.72 | HSCN | 59.08 |
| 21 | Phosphinic acid | 9.642 | 0.59 | $H_3PO_2$ | 66.00 |
| 22 | 3,4-Altrosan | 9.808 | 1.28 | $C_6H_{10}O_5$ | 162.14 |
| 23 | Alpha - pinene | 10.153 | 0.62 | $C_9H_{10}O_3$ | 166.17 |
| 24 | Tetradecanoic acid | 10.242 | 0.40 | $C_{14}H_{28}O_2$ | 228.37 |
| 25 | D-Galactonic acid | 10.675 | 1.07 | $C_6H_{12}O_7$ | 196.16 |
| 26 | Hexanoic acid | 10.853 | 4.29 | $C_6H_{12}O_2$ | 116.16 |
| 27 | Linalool | 11.037 | 0.32 | $C_{10}H_{18}0$ | 154.25 |
| 28 | Undecanoic acid | 11.797 | 1.54 | $C_{11}H_{22}O_2$ | 186.29 |
| 29 | p-Arbutin | 11.842 | 0.63 | $C_{12}H_{16}O_7$ | 272.25 |
| 30 | Scyllo-Inositol | 12.030 | 0.32 | $C_6H_{12}O_6$ | 180.16 |
| 31 | 2,8-Dibenzofurandiamine | 12.175 | 1.45 | $C_{12}H_{10}N_2O$ | 198.22 |
| 32 | Oxirane | 12.375 | 0.42 | $C_2H_4O$ | 44.05 |
| 33 | 2,15-Hexadecanedione | 12.419 | 0.61 | $C_{16}H_{30}O_2$ | 254.41 |
| 34 | 1,2,3,4,5-Cyclopentanepentol | 12.508 | 0.27 | $C_5H_{10}O_5$ | 150.13 |
| 35 | Epi-Inositol | 12.664 | 1.11 | $C_6H_{12}O_6$ | 180.16 |
| 36 | Pentadecanoic acid | 13.197 | 3.85 | $C_{15}H_{30}O_2$ | 242.40 |
| 37 | Beta caryophyllene | 14.138 | 0.85 | $C_{15}H_{24}$ | 204.35 |
| 38 | Octadecanoic acid | 14.475 | 0.90 | $C_{18}H_{36}O_2$ | 284.48 |
| 39 | beta.-Amyrin | 15.052 | 22.00 | $C_{30}H_{50}O$ | 426.72 |
| 40 | Eicosane | 15.308 | 0.52 | $C_{20}H_{42}$ | 282.55 |
| 41 | Ethane | 15.641 | 0.99 | $C_2H_6$ | 30.07 |
| 42 | Pyridine | 16.185 | 0.38 | $C_5H_5N$ | 79.10 |
| 43 | Quercetin | 16.255 | 3.34 | $C_{15}H_{10}O_7$ | 302.24 |
| 44 | alpha.-Amyrin | 16.352 | 1.85 | $C_{30}H_{50}O$ | 426.72 |
| 45 | 15-Hydroxypentadecanoic acid | 16.485 | 2.13 | $C_{15}H_{30}O_3$ | 258.40 |
| 46 | Adamantane | 16.596 | 0.58 | $C_{10}H_{16}$ | 136.23 |
| 47 | 2-Ethylacridine | 16.919 | 0.39 | $C_{15}H_{13}N$ | 207.27 |
| 48 | Luteolin | 17.118 | 0.51 | $C_{15}H_{10}O_6$ | 286.24 |
| 49 | Ethanol | 17.441 | 3.16 | $C_2H_6O$ | 46.07 |
| 50 | Myristoyl chloride | 17.574 | 1.53 | $C_{14}H_{27}ClO$ | 246.82 |
| 51 | Cyclohexaneamine | 17.707 | 0.31 | $C_6H_{13}N$ | 99.17 |
| 52 | Methoxyacetic acid | 18.007 | 0.67 | $C_3H_6O_3$ | 90.08 |
| 53 | Squalene | 18.130 | 0.89 | $C_{30}H_{50}$ | 410.72 |
| 54 | Corydaldine | 18.385 | 0.59 | $C_{10}H_{-13}NO_2$ | 179.22 |
| 55 | Eicosane | 18.652 | 2.50 | $C_{20}H_{42}$ | 282.55 |
| 56 | Cyclotrisiloxane | 18.896 | 0.32 | $H_6Si_3O_3$ | 138.33 |
| 57 | 2H-1-Benzopyran-6-ol | 19.063 | 0.44 | $C_9H_8O_2$ | 148.16 |
| 58 | Propanamide | 19.429 | 0.48 | $C_3H_7NO$ | 73.09 |
| 59 | beta.-Tocopherol | 19.774 | 0.89 | $C_{28}H_{48}O_2$ | 416.68 |
| 60 | gamma.-Tocopherol | 19.918 | 0.43 | $C_{28}H_{48}O_2$ | 416.68 |
| 61 | Eicosane | 20.263 | 0.91 | $C_{20}H_{42}$ | 282.55 |
| 62 | Vitamin E | 20.663 | 3.89 | $C_{29}H_{50}O_2$ | 430.71 |
| 63 | Neoisolongifolene | 22.040 | 1.67 | $C_{15}H_{24}$ | 204.35 |
| 64 | Stigmasterol | 22.229 | 2.60 | $C_{29}H_{48}O$ | 412.69 |
| 65 | Anthracene | 22.662 | 0.38 | $C_{14}H_{10}$ | 178.23 |

flowers" (38). Moreover, lutein is vital in triggering the generation of reactive oxygen species (ROS) (39), indicating the potential of sewage sludge amendment for marigold plants.

Significant translocation factors for nickel (Ni) and cadmium (Cd) from contaminated sites to the roots of two marigold species, *Tagetes patula* and *Tagetes erecta,* were demonstrated (40). A correlation between increased flower yield and the accumulation of heavy metals in marigolds was also noted. These findings are consistent with our results, where higher levels of heavy metals in experimental treatments led to greater yields than the control. Thus, marigolds exhibit potential as a phytoremediation agent for soils contaminated with sewage sludge, while sewage sludge itself could improve the physicochemical characteristics of marigolds".

In summary, this study explored the effects of treated sludge on the phytochemical composition of African Marigold (*Tagetes erecta*) flowers. Using gas chromatography-mass spectrometry (GC-MS) analysis, the marigold flowers treated with treated sludge at 2.5t/ha were analyzed for their phytometabolites. The results revealed an increase in various bioactive compounds in Marigold flowers from plants treated with sludge, including α-Pinene, linalool, β-Caryophyllene, quercetin and luteolin. These compounds are recognized for their antioxidant, anti-inflammatory and antimicrobial properties, indicating potential health benefits associated with Marigold flowers cultivated using treated sludge.

## Conclusion

The current study's findings underscore the potential of treated sludge as a sustainable soil amendment to enhance both plant growth and the phytochemical profile of Marigold flowers. Nonetheless, further research is necessary to elucidate the mechanisms governing the modulation of phytochemical biosynthesis in response to sludge application. Additionally, long-term studies are required to assess this agricultural practice's sustainability and environmental impacts. It is also essential to consider factors such as strain variation, soil composition and environmental conditions to optimize the efficacy of sludge utilization in crop cultivation. Overall, this research enumerates crucial findings on eco-friendly and sustainable farming practices and underscores the utilization of treated sludge as a potent resource for enhancing both the morphological calibre and the bioactive components of African marigolds.

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## Authors' contributions

**KPP** carried out the experiment, took observations and analyzed the data. **SR** guided the research by formulating the concept, helped secure funds and approved the final manuscript. **KLD** contributed by developing the ideas, reviewing the manuscript and helping procure research grants. **SA** contributed by imposing the experiment and helped edit, summarise and revise the manuscript. **MS** helped summarize and revise the manuscript. **MP** helped edit, outline and modify the manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None.

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) did not use AI tools and the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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