**Biocontrol Potential and Volatile Metabolite Profiling of *Beauveria bassiana* against *Rotylenchulus reniformis* and *Fusarium oxysporum* f.sp. *ricini***

**ABSTRACT**

In the present study, the *in‑vitro* growth of Beauveria bassiana was assessed on three media, *viz*., Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), and Oatmeal Agar (OMA). The fungus showed the greatest radial expansion on SDA (7.47 cm), followed by PDA (7.17 cm) and OMA (7.07 cm). Beyond growth performance, the study examined the antimicrobial activity of B. bassiana against the reniform nematode, *Rotylenchulus reniformis,* and the wilt-inducing fungus, Fusarium oxysporum f. sp. ricini, and the organism’s volatile metabolite profile. Bioassays using culture filtrates from the 3 medium against Rotylenchulus reniformis juveniles recorded a highest mortality of 98.67 % at 25 % concentration of the SDA, at 96 h post inoculation. Dual‑culture tests carried out against F. oxysporum f. sp. ricini recorded the highest inhibition (57 %) against B. bassiana grown on SDA. GC‑MS analysis of *B. bassiana* crude extract revealed the presence of 235 compounds belonging to the classes of hydrocarbons, fatty acids and derivatives, esters, alcohols, ketones, aromatics, phenolics, and various heterocyclic/nitrogen-containing molecules. The differences observed among media types for *Beauveria bassiana* growth, nematode mortality, and pathogen inhibition were statistically significant (p < 0.01), confirming the reliability of the experimental outcomes. The findings highlight the significant impact of culture media on the bioactivity and metabolite production of Beauveria bassiana, emphasizing its potential as a versatile biocontrol agent. These results underscore the utility of B. bassiana as a promising component of integrated pest management (IPM) strategies, offering an environmentally sustainable approach to suppress both nematode and fungal pathogens simultaneously. Further studies are necessary to validate these results under field conditions and to develop effective formulations for use in integrated disease management strategies.

Keywords: Beauveria bassiana*,* F. oxysporum f. sp. ricini, GC-MS, Growth, *In vitro* assay, *Rotylenchulus reniformis*, volatile profile.

1. **INTRODUCTION**

**Castor (*Ricinus communis* L.), belonging to the Euphorbiaceae family,** is a vital non-edible oilseed crop predominantly grown in the arid and semi-arid regions of India. Due to its multiple uses in various fields, *viz*., Erisilkworm production, paints, cosmetics, organic fertilizers, pulp and paper industries, lubricants, fuel additives, cosmetics, pharmaceuticals, paints, plastics, and perfumes (Ramanjaneyulu *et al*., 2017), it is also called as Kalpavriksha (Ramanjaneyulu *et al*., 2017). Its resilience under conditions of low rainfall and poor soil fertility makes it highly suitable for dryland agriculture. Castor seeds are notably rich in oil, ranging between 50-55% depending on the variety. The significance of oil lies in its high content of ricinolic acid, which constitutes 85-95% of its composition (Gangaiah, 2008). Wilt, caused by Fusarium oxysporum f.sp. ricini is one of the most widespread and destructive diseases affecting castor. Continuous cultivation of castor in the same fields due to its economic importance has led to the endemic establishment of this disease, becoming a major constraint in castor production across India. Despite high variability in the pathogen, Fusarium oxysporum f.sp. ricini was confirmed as the main causal organism of this vascular disease (Chattopadhyay and Varaprasad, 2001). Yield losses due to *Fusarium* sp. in Castor vary with the growth stage of the crop, reaching up to 77% at flowering, 63% at 90 days after sowing, and 39% during the later stages in secondary branches (Prasad *et al*., 2019). Besides the fungal pathogen, Castor is known to host a range of plant-parasitic nematodes, among which, R. reniformis is considered the most destructive species impacting castor cultivation in India (Patel and Patel, 2009), causing a yield loss of 15% (Kumar *et al*, 2020). This nematode facilitates the entry of secondary fungal infections by compromising root integrity, thereby weakening the plant’s natural defense mechanisms. This makes the host more susceptible to fungal colonization. Conversely, the presence of fungal pathogens enhances nematode survival by producing exudates that attract nematodes or by altering the rhizosphere environment in their favour. Such mutual reinforcement between nematodes and pathogens typically leads to intensified disease symptoms, impaired plant growth, and significant yield reduction (Abawi and Widmer, 2000). The escalating concerns regarding environmental contamination, pest resistance, and health risks associated with chemicals have driven the search for sustainable and biologically safe pest management alternatives. Entomopathogenic fungi (EPF), particularly Beauveria bassiana, have emerged as promising biocontrol agents due to their capacity to infect a wide variety of insect pests, diseases, and nematodes (Zimmermann, 2007; Lacey *et al*., 2015). Traditionally recognized for its insecticidal activity, B. bassiana has also demonstrated nematicidal potential, especially through the production of bioactive secondary metabolites (Quesada-Moraga *et al*., 2009). This entomopathogenic fungus has demonstrated promising antifungal activity, with several strains showing the ability to inhibit the growth of Fusarium oxysporum f.sp. lycopersici (Culebro *et al*., 2017). Besides, the EPF produces a diverse array of toxic secondary metabolites, including fatty acids, hydrocarbons, esters, heterocyclic compounds, and nitrogen-containing molecules, many of which possess strong insecticidal, nematicidal, and antimicrobial properties (Khan *et al*., 2008; Rohlfs and Churchill, 2011). Among these, fatty acid methyl esters (FAMEs), phenolic acids, and pyrazine derivatives have been shown to interfere with nematode sensory and neuromuscular function, thereby limiting their ability to locate and infect host roots. The present study has been carried out to document the growth and *in* *vitro* effectiveness of *B. bassiana* against *R. reniformis* and *F. oxysporum* f.sp. *ricini* and to profile its volatile metabolites through GC-MS analysis.

**2. MATERIALS AND METHODS:**

The study was conducted during October, 2024 – June, 2025 at ICAR-Indian Institute of Oilseeds Research, Hyderabad.

**2.1. Source of *Beauveria bassiana***

 A potent isolate of Beauveria bassiana (ITC 4513) was obtained from the Department of Entomology, ICAR–Indian Institute of Oilseeds Research (IIOR), Hyderabad.

**2.2. Source of *Rotylenchulus reniformis***

 The pure culture of Rotylenchulus reniformis was maintained on Castor plants (GCH-4) under controlled glasshouse conditions (25 ± 2°C) at the Nematology glasshouse, ICAR-IIOR, Hyderabad. The culture was inoculated using a single egg mass of the nematode. Freshly hatched eggs collected from the roots after 45 days were incubated for 7-10 days to obtain J2. Further, one week of incubation converted J2 to J4, which served as the source of inoculum for experimental purposes.

**2.3. Source of *Fusarium oxysporum f.sp. ricini***

*Fusarium* pathogen associated with castor, Fusarium oxysporum f.sp. ricini obtained from the Department of Plant Pathology, ICAR–Indian Institute of Oilseeds Research (ICAR-IIOR), Hyderabad, was used for the present study.

**2.4. Evaluation of growth of *B. bassiana* in different media:**

To assess the growth of *B. bassiana,* three types of culture media viz., Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), and Sabouraud’s Dextrose Agar (SDA), were used (Deb et al., 2019). The required quantity of each medium was prepared and sterilized by autoclaving at 121.6 °C for 15 minutes under 15 *psi* pressure. After sterilization, the medium was melted and aseptically poured into 90 mm diameter Petri plates, dispensing 15 ml per plate under sterilized conditions. The plates were then allowed to solidify, and a 5 mm mycelial disc of 7-day-old B. bassiana was aseptically **transferred to the center of the medium u**sing a sterilized inoculation loop. Plates were incubated under controlled conditions to promote uniform fungal growth and culture establishment. Each treatment was replicated 7 times in a Completely Randomized Design (CRD). Observations on mycelial growth were recorded from three days post-inoculation of fungi and continued until the entomopathogenic fungal growth completely covered the surface of the Petri plate.

**2.5. *In vitro* evaluation ofB. bassianaagainst *R. reniformis***

The *in vitro* evaluation of *B. bassiana,* multiplied on three liquid media *viz*., Potato Dextrose Broth (PDB), Sabouraud Dextrose Broth (SDB), and Oatmeal Broth (OB), was assessed against *R. reniformis*. B. bassiana was inoculated into the sterilized liquid media under aseptic conditions and incubated at 25 ± 1 °C with 50–60% relative humidity for two weeks. After the incubation, fungal mycelia were separated from the culture broth using Whatman No.1 filter paper. The resulting cell-free culture filtrate was collected, and approximately 2 ml of the filtrate of *B. bassiana* multiplied on 3 different broths was added into 40 × 12 mm diameter Petri dishes at varying concentrations (1, 5, 10, 15, and 25%). Around 100 fourth-stage juveniles of R. reniformis were added to each dish. The juveniles added in distilled water served as a control. Each treatment was replicated 3 times in a Completely Randomized Design (CRD). Mortality of the juveniles was assessed at 24h intervals for up to 96h (Youseff *et al*., 2020).

 Juveniles were considered dead if they showed no movement upon gentle stimulation with a fine needle or assumed an open ‘C’ shape.

The percentage of juvenile mortality for each treatment was recorded and corrected for natural mortality observed in the control using Schneider-Orelli’s formula:

Corrected mortality (%) = [(Mortality per cent in treatment – Mortality per cent in the control)/ (100 – Mortality per cent in the control)] × 100.

**2.6. *In vitro* evaluation of *B. bassiana* against *F. oxysporum.* f.sp. *ricini***

 *B. bassiana* grown on PDA, SDA, and OMA media was tested against F. oxysporum f. sp. ricini using a dual culture assay. Cubes of culture medium measuring 5 mm³ were aseptically taken from the actively growing margins of seven-day-old cultures of Fusarium oxysporum f.sp. ricini and B. bassiana, and positioned in a Petri plate at 2 cm from opposite edges, maintaining a distance of 4.5 cm between them. Plates inoculated with the pathogen alone served as a control. The plates were incubated at room temperature for 7 days at 25 ± 2°C. Fungal mycelial growth was measured at 7 days post-incubation, and percent inhibition was calculated (Singh *et al*., 2019). Each treatment was replicated 7 times in a Completely Randomized Design (CRD).

Per cent inhibition over control (I) = [(C–T)/C] X 100 (Dukare *et al*., 2020).

 Where, I = Per cent growth inhibition

 C = Radial growth of the pathogen in control (cm)

 T = Radial growth of the pathogen in treatment (cm)

**2.7. Preparation of crude extracts of *B. bassiana***

 Among the 3 media used, Sabouraud Dextrose broth enhanced the growth of B. bassiana due to which enhancing the antagonistic potential of the fungi. Hence, *B.bassiana* grown in SDB was taken for the preparation of crude extracts. After incubation of the broth at 28 ± 2 °C for 7 days, it was centrifuged at 10,000 rpm for 20 minutes at 4 °C to separate the fungal biomass. The supernatant was collected and filtered through Whatman No. 1 filter paper to remove residual particulates. The clarified filtrate was acidified to pH 2.5 using 1 M hydrochloric acid (HCl), and an equal volume of ethyl acetate was added, and the mixture was vortexed vigorously for 10 minutes to facilitate the extraction of secondary metabolites. The organic phase was concentrated under reduced pressure using a rotary evaporator (D.V.C) and the resultant crude metabolite extract was collected and stored at 4 °C until further use (Chaithra *et al*., 2022).

**2.8. GC-MS analysis of *B. bassiana* crude extract**

 The analysis was performed on an Agilent GC–MS system (Model: CH-GCMSMS02, 8890 GC System coupled with 7000 GC/TQ triple quadrupole) equipped with a DB-5MS capillary column (30 meters in length, 250 μm external diameter, 0.25 μm film thickness). The system was integrated with a Mass Selective Detector (MS-DSQ-II) and operated using Mass Hunter software. Electron ionization at −70 eV was utilized for compound ionization. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Each sample was injected in a 2 μL volume, with the injector maintained at 250°C and the ion source at 200 °C.

 The GC oven temperature was programmed in multiple stages: the run started at 50 °C and held for 1 minute, followed by a temperature increase of 5 °C per minute up to 120 °C, which was maintained for another minute (total time: 16 minutes). The temperature was then ramped at 10 °C per minute to 210 °C, held for 1 minute (total time: 26 minutes), and finally increased at the same rate to 280 °C, with a final hold of 5 minutes (total runtime: 38 minutes). Mass detection was performed within a scan range of 30–900 m/z.

 Identification of compounds was achieved by matching the obtained mass spectra with reference spectra from the NIST and Wiley libraries, which are built into the instrument. Quantification of each compound was performed through peak area normalization, enabling the estimation of their relative abundance within the extract (Duraimurugan *et al*., 2025).

**2.9. Statistical analysis:**

The study comprised data on the growth performance of Beauveria bassiana on various solid media, its *in vitro* nematicidal and fungicidal activities and GC-MS profiling of its crude metabolites. All the experiments were laid out in a Completely Randomized Design (CRD). The data were statistically analyzed using analysis of variance (ANOVA), and treatment means were compared using the critical difference (CD) at a significance level of P ≤ 0.01. Two and three-factor ANOVA were employed to evaluate the growth of B. bassiana across different media and juvenile mortality in response to culture filtrates from three media, respectively. Statistical analyses were performed using the procedures outlined by Gomez and Gomez (1984) and Panse and Sukhatme (1985).

**3. RESULTS AND DISCUSSION**

**3.1. Effect of different media on the growth of *B. bassiana***

 The growth of the B. bassiana was evaluated on three solid media *viz*., Sabouraud Dextrose Agar (SDA), Potato Dextrose Agar (PDA), and Oatmeal Agar (OMA) at intervals of 3, 6, 9, 12, 15, and 19 days post-incubation. **Observations were made on the mycelial growth of the fungi** (Fig.1) and maximum colony growth was observed in Sabouraud Dextrose Agar (SDA) (7.47 cm), followed by Potato Dextrose Agar (PDA) (7.17 cm) and Oatmeal Agar (OMA) (7.07 cm) on 19th day after inoculation **(**Table 1).

  

**OMA**

 **PDA**

 **SDA**

**Fig 1: Influence of different media on the growth of *B. bassiana***

 **Table 1: Mean growth (cm) of Beauveria bassiana on three different media**

|  |  |  |  |
| --- | --- | --- | --- |
| **DAI (Days After Inoculation)** | **Sabouraud Dextrose Agar****(cm)** | **Potato Dextrose Agar****(cm)** | **Oat Meal Agar****(cm)** |
| **3** | 1.27 | 1.23 | 1.17 |
| **6**  | 2.27 | 2.23 | 2.13 |
| **9** | 4.33 | 4.23 | 4.13 |
| **12**  | 5.23 | 5.17 | 5.07 |
| **15**  | 6.43 | 6.17 | 6.07 |
| **19**  | 7.47 | 7.17 | 7.07 |

|  |  |  |  |
| --- | --- | --- | --- |
| **Factors**  | **C.D.** | **SE(d)** | **SE(m)** |
| **M** | 0.039 | 0.019 | 0.014 |
|  **D**  | 0.055 | 0.027 | 0.019 |
| **M×D** | 0.096 | 0.047 | 0.033 |

 **(M: Media, D: Days)**

 The growth assessment of B. bassiana on three solid media revealed significant variations in colony development, with Sabouraud Dextrose Agar (SDA) consistently supporting the most rapid and extensive radial expansion throughout the incubation period. The present findings are consistent with those of Senthamizhselvan *et al*. (2010), who observed that Beauveria bassiana isolates exhibited maximum mycelial growth and sporulation on Sabouraud Dextrose Agar (SDA) compared to Potato Dextrose Agar (PDA), likely due to its optimal carbon-to-nitrogen ratio promoting fungal development. Similarly, Deb *et al.* (2017) reported that SDA provided a nutrient-rich environment favorable for the proliferation of entomopathogenic fungi, attributed to its high dextrose and peptone content, which supports energy metabolism and protein synthesis. However, in contrast, Pandit and Som (1988) identified PDA as a suitable medium for the growth of B. bassiana, suggesting that the starch content in PDA may fulfil the carbon requirements of certain isolates more effectively.

**3.2.**

***In vitro* effect of culture filtrates of *Beauveria bassiana* against reniform nematode juveniles**

 *In vitro* studies carried out with the *B.bassiana* culture filtrate multiplied on three liquid media, *viz*., Sabouraud Dextrose Broth, Potato Dextrose Broth, and Oat Meal Broth, against the J4 of *R.reniformis* revealed a progressive increase in juvenile mortality over time. Among the 3 different liquid media tested, *B.bassiana* multiplied on Sabouraud Dextrose Broth (SDB) resulted in the highest juvenile mortality rate (98.67% in 25% concentration of culture filtrate at 96h post-incubation), followed by Potato Dextrose Broth (PDB) and Oatmeal Broth (OMB) with the mortality rate of 98 and 70.13% at the same concentration and exposure time (Fig.2; Table 2).



**Fig. 2 Effect of *B.bassiana* culture filtrate on nematode juveniles**

 According to Liu *et al*. (2008), Beauveria bassiana cultured (1%) cultured in Czapek-Dox Broth recorded the strong nematicidal activity of 96.39% against the juveniles of Meloidogyne hapla, which was comparable to the chemical nematicide Aldicarb, supporting its potential as an effective biocontrol agent. B. bassiana cultured in Sabouraud Dextrose Yeast Broth, when applied at the highest concentration (100%), effectively reduced second-stage juveniles (J2) of Meloidogyne incognita by 86.4% in soil and 85.7% in roots, indicating strong nematicidal potential (Youseff *et al*., 2020).

**Table 2: Effect of *B. bassiana* grown on different media on the juvenile mortality of *R. reniformis***

|  |  |  |
| --- | --- | --- |
| **Media** | **Time (h)** |  **Concentration** |
| **1%** | **5%** | **10%** | **15%** | **25%** |
| **SDB** | **24** | **27.33** | **31.33** | **35.67** | **36.33** | **37.67** |
| **48** | **43** | **50.67** | **55.33** | **65.33** | **68.33** |
| **72** | **53.67** | **60.33** | **59.33** | **72.33** | **88.33** |
| **96** | **61.33** | **65.33** | **70.33** | **82.33** | **98.67** |
| **PDB** | **24** | **23.33** | **24** | **26.67** | **32.0** | **33.07** |
| **48** | **35.33** | **44.33** | **45.33** | **50.67** | **62.33** |
| **72** | **48.33** | **51.5** | **53.67** | **62.33** | **84.67** |
| **96** | **51.67** | **57.33** | **67.67** | **73.0** | **98** |
| **OMB**  | **24** | **20.67** | **28.67** | **32.17** | **33.0** | **33.5** |
| **48** | **29.33** | **31.33** | **34.0** | **44.33** | **46.17** |
| **72** | **36.33** | **39.33** | **43.33** | **59.33** | **70.13** |
| **96** | **44.67** | **52.33** | **53.33** | **69.5** | **86.67** |
| **Control** | **24** | **0.0** |
|  | **48** | **0.3** |
|  | **72** | **1.3** |
|  | **96** | **2.7** |

|  |  |  |  |
| --- | --- | --- | --- |
| **Factors** | **C.D.** | **SE (d)** | **SE (m)** |
| **M** | **0.586** | **0.296** | **0.209** |
| **D** | **0.757** | **0.382** | **0.270** |
| **M×D** | **1.311** | **0.662** | **0.468** |
| **C** | **0.677** | **0.342** | **0.242** |
| **M×C** | **1.173** | **0.592** | **0.419** |
| **D×C** | **1.514** | **0.765** | **0.541** |
| **M×D×C** | **2.623** | **1.324** | **0.937** |

 **(M: Media, D: Days, C: Concentration)**

**c. *In vitro* effect of *Beauveria bassiana* grown on different media against *F. oxysporum* f. sp. *ricini***

Results of the dual culture assay revealed a differential inhibition of *Fusarium oxysporum* f.sp. *ricini* by *Beauveria bassiana,* grown in the three tested media. The most pronounced suppression of radial growth of the fungi was observed with *B.bassiana* grown on Sabouraud Dextrose Agar (SDA) (3.44 cm) followed by Potato Dextrose Agar (PDA) (3.74 cm) and Oatmeal Agar (OMA) (5 cm) after 7 days post-inoculation (Fig.3; Table 3). These findings proved that the antagonistic activity of the entomopathogenic fungus varies with the nutrient composition of the medium.



 **Fig 3: *In vitro* effect of *B.bassiana* grown on different media against *F.oxysporum* f.sp. *ricini***

The dual culture assay demonstrated that SDA supported the greatest inhibition of F. oxysporum f.sp. ricini, followed by PDA and OMA. These results are supported by Deb *et al*. (2017), who noted superior antagonistic and growth-enhancing effects of B. bassiana cultured on SDA compared to OMA. These results suggest that nutrient availability in SDA promotes the synthesis of antifungal substances. Culebro *et al*. (2017) reported that in dual culture assays, the Beauveria bassiana strain 1215 exhibited strong antagonistic activity against Fusarium oxysporum f.sp. lycopersici race 3, achieving 72% mycelial growth inhibition. The authors observed the interaction led to noticeable mycelial collapse of the pathogen, indicating effective suppression through direct fungal interaction.

**Table. 3. Effect of *B. bassiana* grown on different media on the mycelial growth of *F.oxysporum* f. sp. *ricini***

|  |  |  |
| --- | --- | --- |
| **Media** | **Mean mycelial growth\* (cm)** | **Percent Inhibition over control** |
| **Sabouraud Dextrose Agar** | 3.44 | 57.0 |
| **Potato Dextrose Agar** | 3.74 | 53.25 |
| **Oat Meal Agar** | 5.0 | 37.50 |
| **Control** | 8.0 | 0 |
| **CD (p≤0.01)** | 0.28 | 0.98 |
| **SE (d)** | 0.09 | 0.46 |
| **SE (m)** | 0.13 | 0.33 |

 **\*Mycelial growth recorded at 7 days post inoculation.**

**e. Profiling of volatile compounds of *B.bassiana* using GC-MS analysis**

 Gas Chromatography-Mass Spectrometry (GC-MS) analysis of ethyl acetate of Beauveria bassiana resulted in the detection of a diverse array of **235 compounds** (Table 5). The majority of these compounds belonged to the classes of **hydrocarbons, fatty acids and their derivatives, esters, alcohols, ketones, aromatic compounds, phenolics**, as well as **heterocyclic and nitrogen-containing compounds** (supplementary table 1). The abundantly reported compounds were the **fatty acid methyl esters**, such as methyl palmitate, methyl oleate, and methyl linoleate, and these compounds were known to exhibit antimicrobial, antifungal, and insecticidal activities**.** Besides, the presence of phthalic acid esters and related aromatic esters suggests their possible contribution to the bioactivity and structural complexity of the secondary metabolite profile.

 Chromatographic profiling of crude metabolites extracted from *Beauveria bassiana* reported several TIC peaks, indicating the abundance of the bioactive compounds. The 21 predominant peaks were analysed, and the compounds present in the peak with their retention time, % area, class, and properties were presented in Table 4.

 The GC–MS analysis of Beauveria bassiana ethyl acetate extracts in the current study revealed that fatty acid methyl esters—particularly hexadecanoic acid methyl ester, 9,12‑octadecadienoic acid methyl ester (E,E)-, and tetradecanoic acid 12‑methyl, methyl ester—were among the most abundant compounds. Supporting this, Abdullah *et al*. (2020) identified similar methyl esters using GC–MS in B. bassiana extracts, where hexadecanoic acid methyl ester (14.96%), n‑hexadecanoic acid (21.14%), and 9,12‑octadecadienoic acid methyl ester (11.98%) comprised the major peaks. These compounds have been previously associated with larvicidal, nematicidal, and general pesticidal activity.

 A comprehensive GC–MS analysis conducted by El-Shazly *et al*. (2025) on Beauveria bassiana ethyl acetate fractions revealed the presence of several dominant bioactive constituents, including methyl esters of palmitic acid, (Z)-9-octadecenoic acid, cis-5-eicosenoic acid, and dioctyl phthalate. These compounds are well-documented for their pesticidal activities, particularly against insect pests and plant-parasitic nematodes. Fatty acid methyl esters, such as those derived from palmitic and oleic acids, exert their toxic effects by compromising cell membrane integrity and disrupting essential enzymatic functions in the target organisms. Furthermore, dioctyl phthalate contributes to the overall chemical complexity and may act synergistically with fatty acids to enhance the bioefficacy of the fungal extract. The identification of these compounds reinforces the potential of B. bassiana as a rich source of secondary metabolites for use in sustainable pest and nematode management strategies.

**4. CONCLUSION**

The present study demonstrates that Beauveria bassiana exhibits optimal growth and enhanced bioactivity when cultured on Sabouraud Dextrose Agar, which significantly influenced its antagonistic effects against Rotylenchulus reniformis and Fusarium oxysporum f. sp. ricini. The high juvenile mortality and fungal growth inhibition observed, along with the identification of a diverse range of volatile bioactive compounds through GC-MS analysis, underscore the strong biocontrol potential of B. bassiana. These findings not only confirm the critical role of culture media in modulating the EPF efficacy but also highlight its promise as a sustainable and effective component in integrated pest and disease management. Further validation under field conditions and development of stable formulations will be key to harnessing its full potential for agricultural applications.



 Fig 4. Chromatographic profiling of the crude metabolites from *Beauveria bassiana*

**Table 4. Identification, classification, and characteristics of compounds represented by major GC-MS chromatogram peaks**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** |  **RT** | **Peak observed** | **Compound** | **Structure** | **% Area** |  **Class** | **Antimicrobial properties** |  **Reference** |
| **1.** | **4.21** |  | **1-Butanol,3-methyl-acetate** |  | **4.05** | **Carboxylic Esters** | **Against bacteria and fungi** | **Fialho *et al.*, 2011.** |
| **2.** | **6.39** |  | **Decane** |  | **4.86** | **Alkane** |  **-** |  **-** |
| **3.** | **7.72** |  | **Nonane,4,5-dimethyl-** |  | **5.72** | **Alkane** |  **-** |  **-** |
| **4.** | **11.19** |  | **Cyclododecane** |  | **9.05** | **Cycloalkane** | **Against bacteria and fungi** | **Sahu *et al*., 2021** |
| **5.** | **12.83** |  | **Benzene,1,3-bis(1,1-dimethylethyl)-** |  | **9.73** | **Alkylbenzene** | **Against bacteria** | **Faridha *et al*., 2016** |
| **6.** | **13.53** |  | **Pentadecane** |  | **7.61** | **Alkane** | **Against protozoa** | **Bruno *et al*., 2015** |
| **7.** | **15.41** |  | **Benaldehyde, 4-nitro** |  | **100** | **Aromatic aldehyde** | **Against bacteria** | **Umar *et al*., 2025** |
| **8.** | **16.58** |  | **Cyclotetradecane** |  | **18.52** | **Cycloalkane** | **Against bacteria** | **Naz *et al*. 2020.** |
| **9.** | **19.18** |  | **2,4-Di-tert-butyl phenol** |  | **17.90** | **Phenol** | **Against fungi** | **Varsha *et al*., 2015** |
| **10.** | **20.45** |  | **1-Hexadecanol** |  | **14.35** | **Alcohol** | **Against bacteria** | **Togashi *et al*., 2007** |
| **11.** | **21.98** |  | **Heneicosane** |  | **6.01** | **Alkane** | **Against bacteria** | **Wijayanti *et al*., 2022** |
| **12.** | **22.97** |  | **1-Octadecene** |  | **10.43** | **Alkene** | **Against *E.coli*** | **Ngema *et al*., 2023** |
| **13.** | **23.73** |  | **Cyclo(L-propyl-L-valine)** |  | **19.18** | **Cyclic dipeptide** | **Against bacteria** | **Ren *et al*., 2022.** |
| **14.** | **24.43** |  | **Hexadecanoic acid, methyl ester** |  | **11.14** | **FAME (Fatty acid methyl ester)** | **Against bacteria and fungi** | **Agoramoorthy *et al*., 2007** |
| **15.** | **25.31** |  | **Pyrrolo (1,2a) pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-** |  | **55.28** | **Cyclic peptide** | **Against bacteria and fungi** | **Al-Askar *et al*., 2024** |
| **16.** | **26.33** |  | **9-Octadecenoic acid (Z)-, methyl ester**  |  | **9.51** | **FAME (Fatty acid methyl ester)** | **Against bacteria** | **Marusich *et al*., 2020** |
| **17.** | **27.23** |  | **Octadecanoic acid** |  | **39.36** | **Fatty acid** | **Against bacteria** | **Yoon *et al*., 2018** |
| **18.** | **28.44** |  | **2,5-Piperazinedone, 3,6-bis (2-methylpropyl)-** |  | **14.48** | **Cyclic dipeptide** | **Against bacteria** | **Driche *et al*., 2024** |
| **19.** | **29.77** |  | **Pyrrolo (1,2-a) pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)** |  | **67.47** | **Cyclic dipeptide** | **Against bacteria and fungi** | **Kiran *et al*., 2018** |
| **20.** | **31.04** |  | **Cycloteradecane** |  | **4.76** | **Cycloalkane** | **Against bacteria and fungi** | **Naz *et al*., 2020** |
| **21.** | **32.31** |  | **Chloramphenicol** |  | **2.89** | **Amphenicols** | **Against bacteria** | **Schwarz *et al*., 2004** |

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1. No AI Technlogies were used .

2.

3.

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**(Supplementary TABLE 1):** **TABLE 5**

**TABLE 5 Profiling of volatile metabolites of *Beauveria bassiana* by GC-MS analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No** | **RT** | **Name of the compound** |  **Formula** | **Classification** |
| 1 | 3.28 | Octane | C8H18 | Hydrocarbon  |
| 2 | 3.35 | 3-Hydroxy-azetidine-1-carboxylic acid, methyl ester | C5H9NO3 |  N- containing heterocyclic compound |
| 3 | 3.44 | Propane, 1,1-dimethoxy-2-methyl- | C6H14O2 | Aliphatic Ether  |
| 4 | 3.94 | Octanoic acid, 2-methyl-, methyl ester | C10H20O2 | Aliphatic Ester |
| 5 | 4.21 | 1-Butanol, 3-methyl-, acetate | C7H14O2 | Organic Ester |
| 6 | 4.62 | Methylal | C3H8O2 | Diether |
| 7 | 5.06 | Pyrazine, 2,6-dimethyl- | C6H8N2 | Aromatic N-containing heterocycle |
| 8 | 5.56 | Vanadyl phthalocyanine | C32H16N8OV | Organometallic compound |
| 9 | 5.89 | Pentane, 2,2,4,4-tetramethyl- | C9H20 | Hydrocarbon  |
| 10 | 6.03 | Benzaldehyde | C7H6O | Aromatic Aldehyde |
| 11 | 6.07 | (1H) Pyrrole-3-carbonitrile, 2-methyl- | C6H6N2 | Heterocyclic aromatic compound |
| 12 | 6.13 | 1,5-Pentanediol, O,O'-di(3-methylbut-2-enoyl)- | C15H24O4 | Diester |
| 13 | 6.39 | Decane | C10H22 | Hydrocarbon  |
| 14 | 6.40 | Cyclotetrasiloxane, octamethyl- | C8H24O4Si4 | Organosilicon compound |
| 15 | 6.70 | 2,2-Dimethylpropanoic anhydride | C10H18O3 | Acid anhydried, Aliphatic compound |
| 16 | 6.83 | 3-Amino-s-triazole | C2H4N4 | Organic Heterocyclic Compound |
| 17 | 6.96 | Benzene, 1,2,4-trimethyl- | C9H12 | Aromatic Hydrocarbon |
| 18 | 7.14 | Butanamide, 3-methyl- | C5H11NO | Organic Amide |
| 19 | 7.26 | Indane | C9H10 | Heterocyclic aromatic compound |
| 20 | 7.53 | 4-Amino-2,6-dihydroxypyrimidine | C4H5N3O2 | Heterocyclic compound , pyramidine derivative |
| 21 | 7.95 | N-Methyltaurine | C3H9NO3S | Organic Nitrogen compound |
| 22 | 8.33 | Silane, (4-methoxyphenyl) trimethyl- | C10H16OSi | Organosilicon compound |
| 23 | 8.35 | Cyclopentane, 1,1,3,4-tetramethyl-, trans- | C9H18 | Hydrocarbon  |
| 24 | 8.55 | Pyrazine, 2,6-diethyl- | C8H12N2 | Heterocyclic compound , pyrazine derivative |
| 25 | 8.74 | Benzaldehyde, 4-methyl- | C8H8O | Aromatic Aldehyde |
| 26 | 8.82 | Nonane, 4,5-dimethyl- | C11H24 | Hydrocarbon  |
| 27 | 8.96 | 2-Furanmethanol, tetrahydro-, acetate | C7H12O3 | Organic Ester |
| 28 | 9.30 | Benzene, 1,2,4,5-tetramethyl- | C10H14 | Aromatic Hydrocarbon |
| 29 | 9.55 | 2-Methylheptanoic acid | C8H16O2 | Fatty acid |
| 30 | 9.78 | 1,2-Benzenediol, O-(4-methylbenzoyl)-O'-(2- | C22H15F3O4 | Aromatic Ester |
| 31 | 10.00 | 2-Azetidinone, 3,3-dimethyl-4-phenyl-1-propyl- | C14H19NO | Heterocyclic Organic compound |
| 32 | 10.32 | 2-(5-Phenyl- [1,3,4] thiadiazol-2-yl)- | C20H11N3O2S | Heterocyclic compound |
| 33 | 10.33 | 1-Methyl-5-fluorouracil | C5H5FN2O2 | Fluoropyramidine derivative |
| 34 | 10.33 | Cyclopentasiloxane, decamethyl- | C10H30O5Si5 | Organosilicon compound |
| 35 | 10.37 | Silane, cyclohexyldimethoxymethyl- | C9H20O2Si | Organosilicon compound |
| 36 | 10.37 | 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- | C6H8O4 | Heterocyclic compund |
| 37 | 10.42 | Hydrogen isocyanate | CHNO | Isocyanate inorganic compound |
| 38 | 10.81 | N-Ethylamphetamine, N-trimethylsilyl- | C14H25NSi | Organosilicon compound |
| 39 | 10.98 | 1-Benzocyclobutenecarbonitrile | C9H7N | Aromatic compound |
| 40 | 11.19 | Cyclododecane | C12H24 | Hydrocarbon  |
| 41 | 11.29 | d-Proline, N-methoxycarbonyl-, dodecyl ester | C19H35NO4 | Aminoacid derivative |
| 42 | 11.76 | 3,5-Diamino-1,2,4-triazole | C2H5N5 | Heterocyclic aromatic compound |
| 43 | 11.77 | Butane, 2,2-dimethyl- | C6H14 | Hydrocarbon  |
| 44 | 11.98 | Ethyl 4-(ethyloxy)-2-oxobut-3-enoate | C8H12O4 | Organic compound |
| 45 | 12.04 | Benzene, 1-methyl-3-nitro- | C7H7NO2 | Nitroaromatic compound |
| 46 | 12.19 | Benzaldehyde, 2-ethyl- | C9H10O | Aromatic Aldehyde |
| 47 | 12.25 | 1H-Pyrazolo[3,4-d] pyrimidin-4-amine | C5H5N5 | Heterocyclic compound |
| 48 | 12.38 | n-Pentyl methylphosphonofluoridate | C6H14FO2P | Organophosphorus compound |
| 49 | 12.57 | Decane, 2,4-dimethyl- | C12H26 | Hydrocarbon  |
| 50 | 12.83 | Benzene, 1,3-bis(1,1-dimethylethyl)- | C14H22 | Aromatic compound |
| 51 | 12.86 | 4-Piperidinone, 1-methyl- | C6H11NO | Heterocyclic compound |
| 52 | 13.37 | 3H-1,2-Dithiol-3-one, 4-methyl- | C4H4OS2 | Heterocyclic compound |
| 53 | 13.53 | Pentadecane | C15H32 | Hydrocarbon  |
| 54 | 13.74 | Cyclobutylamine, N-trimethylacetyl- | C9H17NO | Amide derivative |
| 55 | 13.82 | Benzeneacetic acid | C8H8O2 | Aromatic carboxylic acid |
| 56 | 13.86 | 1-Aminocyclopentanecarboxylic acid, N- | C18H33NO4 | Aminoacid derivative |
| 57 | 14.20 | Cyclopentanecarboxamide, N-(4-fluorophenyl)- | C12H14FNO | Amide derivative |
| 58 | 14.42 | 2-Hepten-4-one, 6-methyl- | C8H14O | Ketone |
| 59 | 14.54 | Pyridine, 2,2'-methylenebis- | C11H10N2 | Aromatic Heterocyclic compound |
| 60 | 14.93 | Cyclohexasiloxane, dodecamethyl- | C12H36O6Si6 | Cyclic siloxane compound |
| 61 | 15.12 | Acetamide, 2-phenyl-N-(2-phenylethyl)-N-nonyl- | C25H35NO | Amide compound |
| 62 | 15.41 | Benzaldehyde, 4-nitro- | C7H5NO3 | Aromatic Aldehyde |
| 63 | 15.62 | 9H-Carbazole, 9-ethyl- | C14H13N | Heterocyclic aromatic compound |
| 64 | 16.84 | Demethoxyencecalinol | C13H16O2 | Phenolic organic compound |
| 65 | 16.87 | Benzenemethanol, 3-(dimethylamino)- | C9H13NO | Aromatic alcohol |
| 66 | 17.04 | Cyclododecanol | C12H24O | Aliphatic alcohol |
| 67 | 17.11 | Pentobarbital | C11H18N2O3 | Diketone |
| 68 | 17.12 | Propanoic acid, ethenyl ester | C5H8O2 | Organic Estter |
| 69 | 17.21 | 3,3-Tetramethyleneglutaric anhydride | C9H12O3 | Aliphatic compound |
| 70 | 17.24 | 3-phenyl-oxindole | C14H11NO | Heterocyclic compound |
| 71 | 17.89 | Benzenepropanenitrile | C9H9N | Organic nitrile compound |
| 72 | 18.47 | Formic acid, decyl ester | C11H22O2 | Ester |
| 73 | 18.82 | 5-Amino-2-methoxyphenol | C7H9NO2 | Aromatic compound |
| 74 | 19.05 | Methane, isothiocyanato- | C2H3NS | Organosulphur compound |
| 75 | 19.11 | N-Ethyl-2-furancarbothioamide | C7H9NOS | Organic sulphur compound |
| 76 | 19.18 | 2,4-Di-tert-butylphenol | C14H22O | Phenolic compound |
| 77 | 19.27 | 1-Propanol, 2-(2-hydroxypropoxy)- | C6H14O3 | Glycol Ether |
| 78 | 19.35 | Ethanedioic acid, dimethyl ester | C4H6O4 | Ester |
| 79 | 19.35 | Dodecanoic acid, methyl ester | C13H26O2 | Fatty acid Ester |
| 80 | 19.45 | Pyridin-2,6-diol, diacetate | C9H9NO4 | Pyridine based Ester |
| 81 | 19.54 | 5-Fluoro-2-trifluoromethylbenzoic acid, 4- | C14H7F4NO4 | Fluorinated aromatic compound |
| 82 | 19.54 | 2-(p-Fluorophenyl)-1-methylbenzimidazole | C14H11FN2 | Heterocyclic aromatic compound |
| 83 | 19.61 | 2-Butenedioic acid (Z)-, dibutyl ester | C12H20O4 | Ester |
| 84 | 19.68 | Pentanedioic acid, (2,4-di-t-butylphenyl) mono- | C19H28O4 | Aromatic compound  |
| 85 | 19.71 | Benzoic acid, 4-(4-ethylcyclohexyl)-, 4-butoxy-2,3- | C27H30N2O3 | Organic compound |
| 86 | 19.74 | Sulfurous acid, dodecyl pentyl ester | C17H36O3S | Organosulphur compound |
| 87 | 19.76 | 2,6-Pyridinedicarboxylic acid, diphenethyl ester | C23H21NO4 | Heterocyclic aromatic compound |
| 88 | 19.94 | 9H-Pyrrolo[1,2-a] indol-9-one, 2-methyl- | C12H9NO | Heterocyclic compound |
| 89 | 20.08 | Benzene, (2-chloroethyl)- | C8H9Cl | Organochlorine compound |
| 90 | 20.20 | Dodecanoic acid | C12H24O2 | Organic compound |
| 91 | 20.40 | 1-Hexadecanol | C16H34O | Organic compound |
| 92 | 20.43 | Trimethyl(n-octyl) silane | C11H26Si | Organosilicon compound |
| 93 | 20.58 | 2-Tetradecanol | C14H30O | Organic compound |
| 94 | 20.65 | Carbamic acid, methylphenyl-, ethyl ester | C10H13NO2 | Organic compound |
| 95 | 20.70 | Ethanone, 1-(2-hydroxy-4-methoxyphenyl)- | C9H10O3 | Aromatic organic compound |
| 96 | 20.72 | 2-Bromo-4,6-di-tert-butylphenol | C14H21BrO | Aromatic organic compound |
| 97 | 20.73 | 2-Thiophenylacetic acid, 2,2,2-trifluoroethyl ester | C8H7F3O2S | Organic compound |
| 98 | 20.79 | 9H-Carbazole-3,6-diamine | C12H11N3 | Heterocyclic aromatic compound |
| 99 | 20.82 | Naphthalene, decahydro-1,6-dimethyl- | C12H22 | Organic compound |
| 100 | 20.87 | 1-Benzyl-2-(trifluoromethyl)aziridine | C10H10F3N | Fluorinated aromatic aziridine |
| 101 | 21.02 | Benzophenone | C13H10O | Organic compound |
| 102 | 21.14 | [2-(4-Benzyloxy-phenyl)-1-carbamoyl-ethyl]- | C21H26N2O4 | Aromatic organic compound |
| 103 | 21.51 | Borane, diethyl(decyloxy)- | C14H31BO | Organoboron compound |
| 104 | 21.68 | Sulfurous acid, 2-ethylhexyl undecyl ester | C19H40O3S | Organic sulphur compound |
| 105 | 21.68 | Phenol, 2,4-di-t-butyl-6-nitro- | C14H21NO3 | Organic compound |
| 106 | 21.79 | Dodecyl acrylate | C15H28O2 | Organic compound |
| 107 | 21.85 | Ethylamine, N-heptyl-N-octyl-2-(2-thiophenyl)- | C21H39NS | Organic Nitrogen compound |
| 108 | 21.90 | Silane, dimethoxydimethyl- | C4H12O2Si | Organosilicon compound |
| 109 | 22.08 | Heneicosane | C21H44 | Organic compound |
| 110 | 22.12 | Hexestrol | C18H22O2 | Aromatic organic compound |
| 111 | 22.16 | Metoclopramide | C14H22ClN3O2 | Organic compound |
| 112 | 22.18 | Methyl tetradecanoate | C15H30O2 | Organic compound |
| 113 | 22.42 | 3,5-Dimethyldodecane | C14H30 | Ester |
| 114 | 22.44 | 3-Cyclopentylpropionamide, N-(2-fluorophenyl)- | C14H18FNO | Amide |
| 115 | 22.46 | Hexestrol dimethyl ether | C20H26O2 | Ether  |
| 116 | 22.47 | p-(Benzylideneamino)phenol | C13H11NO | Organic compound |
| 117 | 22.58 | 1,2-Benzenediol, o-(4-methoxybezoyl)-o'-(5- | C19H19ClO5 | Aromatic compound |
| 118 | 22.61 | 2,5-Piperazinedione, 3-methyl-6-(1-methylethyl)- | C8H14N2O2 | Heterocyclic organic compound |
| 119 | 22.67 | Oxirane, 2-(4-nitrophenyl)- | C8H7NO3 | Aromatic organic compound |
| 120 | 22.70 | 3,5-di-tert-Butyl-4-hydroxybenzaldehyde | C15H22O2 | Organic compound |
| 121 | 22.76 | Tetradecanoic acid | C14H28O2 | Aliphatic organic compound |
| 122 | 22.76 | 2-Cyano-2-O-fluorosulfatofluoropropane | C4F7NO3S | Organofluorine cmpound |
| 123 | 22.82 | Benzeneacetic acid, 4-hydroxy- | C8H8O3 | Organic compound |
| 124 | 22.91 | l-Leucine, n-propargyloxycarbonyl-, octyl ester | C18H31NO4 | Organic compund |
| 125 | 22.97 | 1-Octadecene | C18H36 | Hydrocarbon  |
| 126 | 23.04 | Octadecane | C18H38 | Staurated Hydrocarbon |
| 127 | 23.13 | Cyclopropane, 1-methyl-1-(1-methylethyl)-2-nonyl- | C16H32 | Organic compound |
| 128 | 23.27 | N-Phenyl-5,6,7,8-tetrahydroquinazolin-2-amine | C14H15N3 | Heterocyclic amine |
| 129 | 23.31 | 1,2-Dihydro-4-methyl-6-nitro-2-oxoquinoline | C10H8N2O3 | Nitrogenous Heterocyclic compound  |
| 130 | 23.31 | 3,5-di-tert-Butyl-4-hydroxyacetophenone | C16H24O2 | Organic compound |
| 131 | 23.35 | Cycloheptanemethanol | C8H16O | Cyclic alcohol |
| 132 | 23.38 | Benzothiazole-5-carboxylic acid | C8H5NO2S | Aromatic organic compound |
| 133 | 23.38 | Benzoic acid, 4-[(1,3-dioxobutyl) amino]- | C11H11NO4 | Aromatic compound |
| 134 | 23.58 | Nonane, 1-iodo- | C9H19I | Organic compound |
| 135 | 23.58 | 3-Ethyl-2,6,10-trimethylundecane | C16H34 | Organic compound |
| 136 | 23.59 | (3S,6S)-3-Butyl-6-methylpiperazine-2,5-dione | C9H16N2O2 | Organic compound |
| 137 | 23.64 | 1H-1,2,4-Triazole | C2H3N3 | Azole |
| 138 | 23.65 | N-Norvaline, n-propargyloxycarbonyl-, octyl ester | C17H29NO4 | Ester |
| 139 | 23.86 | Phthalic acid, butyl 2-pentyl ester | C17H24O4 | Aromatic Ester |
| 140 | 23.95 | 2-Octene, 4-ethyl-, (E)- | C10H20 | Organic compound |
| 141 | 23.95 | Cyclohexane, ethyl- | C8H16 | Organic compound |
| 142 | 23.96 | 5-Methyl-3-(1H-pyrazol-3-yl)-1,2,4-oxadiazole | C6H6N4O | Pyrazole derivative |
| 143 | 24.03 | Phosphorus pentafluoride | F5P | Phosphorus compound |
| 144 | 24.05 | Phenadoxone | C23H29NO2 | Ether  |
| 145 | 24.17 | 2-Butyl-3,4,5,6-tetrahydropyridine | C9H17N | Heterocyclic organic compound |
| 146 | 24.43 | Hexadecanoic acid, methyl ester | C17H34O2 | Fatty acid ester |
| 147 | 24.59 | Cyclobutanecarboxamide, N-(3-methylphenyl)- | C12H15NO | Amide |
| 148 | 24.61 | N-Ethyl-5-propyl-5-nonanamine | C14H31N | Alkyl substituted amine |
| 149 | 24.63 | 2,5-Thiophenedicarboxaldehyde | C6H4O2S | Aromatic aldehyde |
| 150 | 24.70 | L-Proline, ethyl ester | C7H13NO2 | Ester |
| 151 | 24.79 | Ethanol, 2-[4-(1,1-dimethylethyl)phenoxy]- | C12H18O2 | Aromatic ether |
| 152 | 24.91 | Ethanol, 2-[2-(4-nonylphenoxy)ethoxy]- | C19H32O3 | Aromatic ether |
| 153 | 24.92 | 1-(4-Benzylpiperidin-1-yl)-2-(3,4- | C22H25NO4 | Aromatic organic compound |
| 154 | 25.06 | Dibutyl phthalate | C16H22O4 | Ester |
| 155 | 25.16 | 2,6-Difluoro-3-methylbenzoic acid, 2,3- | C14H8Cl2F2O2 | Fluorobenzene derivative |
| 156 | 25.18 | 2-Acetyl-3,5-dimethylbenzo(b)thiophene | C12H12OS | Thioether |
| 157 | 25.22 | N,3-Diethyl-3-nonanamine | C13H29N | Amine |
| 158 | 25.30 | n-Hexadecanoic acid | C16H32O2 | Fatty acid |
| 159 | 25.31 | Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(2- | C11H18N2O2 | Heterocyclic compound |
| 160 | 25.33 | Benzoic Acid, TBDMS derivative | C13H20O2Si | Silicon-containing compound |
| 161 | 25.33 | Acetamide, 2-(4-hydroxy-3-methoxyphenyl)- | C9H11NO3 | Aromatic compound |
| 162 | 25.36 | 1,3-Diphenyl-4H-1,2,4-triazoline-5-thione | C14H11N3S | Heterocyclic organic compound |
| 163 | 25.39 | Acetamide, N-9-phenanthrenyl- | C16H13NO | Amide |
| 164 | 25.41 | Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-, | C17H25NO2 | Organic compound |
| 165 | 25.44 | 4-Benzyloxy-3-methoxy-2-nitrobenzoic acid | C15H13NO6 | Aromatic organic compound |
| 166 | 25.48 | Vanillin, butyl ether | C12H16O3 | Organic compound |
| 167 | 25.73 | 1,3-Benzenedicarboxylic acid, 5-(1,1-dimethylethyl)- | C12H14O4 | Aromatic organic compound |
| 168 | 25.76 | Benzothiazole, 2-(4-tert-butylphenyl)- | C17H17NS | Heterocyclic organic compound |
| 169 | 25.81 | Dispiro[1,3-dioxolane-2,2'-bicyclo[2.2.1]heptane- | C14H22O4 | Heterocyclic organic compound |
| 170 | 25.83 | 5-Methyl-5-triazolo(1,5-a)pyrimidine | C6H6N4 | Heterocyclic organic compound |
| 171 | 26.14 | n-Pentadecanol | C15H32O | Alcohol |
| 172 | 26.26 | 9,12-Octadecadienoic acid (Z,Z)-, methyl ester | C19H34O2 | Fatty acid methyl ester  |
| 173 | 26.33 | 9-Octadecenoic acid (Z)-, methyl ester | C19H36O2 | Fatty acid methyl ester  |
| 174 | 26.39 | Octadecanenitrile | C18H35N | Fatty Nitrile |
| 175 | 26.61 | Methyl stearate | C19H38O2 | Fatty acid methyl ester  |
| 176 | 26.71 | Fumaric acid, isobutyl pent-4-en-2-yl ester | C13H20O4 | Ester |
| 177 | 26.71 | Hexadecane, 2,6,10,14-tetramethyl- | C20H42 | Organic compound |
| 180 | 26.72 | di-n-Undecylamine | C22H47N | Amine |
| 181 | 26.85 | Phthalic acid, monoamide, N,N'-diethyl-N,N'- | C24H24N2O2 | Amide |
| 182 | 26.89 | 6-Octadecenoic acid | C18H34O2 | Organic compound |
| 183 | 26.90 | 1-Butanone, 1-(2-furanyl)- | C8H10O2 | Organic compound |
| 184 | 26.97 | 3,4-Methylenedioxybnezophenone | C14H10O3 | Organic compound |
| 185 | 27.05 | Ethylene, 1-nitro-2-[3-benzyloxyphenyl]- | C15H13NO3 | Nitroalkene |
| 186 | 27.07 | Phosphoric acid, diethyl pentyl ester | C9H21O4P | Phosphoric acid ester |
| 187 | 27.22 | Methyl methylphosphonofluoridate | C2H6FO2P | Organophosporus compound |
| 190 | 27.23 | Octadecanoic acid | C18H36O2 | Organic compound |
| 191 | 27.33 | Behenic alcohol | C22H46O | Organic compound |
| 192 | 27.38 | Docosane | C22H46 | Organic compound |
| 193 | 27.40 | Hexadecanamide | C16H33NO | Amide |
| 194 | 27.62 | Phosphinic acid, (1,1-dimethylethyl)[4-(1,1- | C14H23O2P | Organic compound |
| 195 | 27.69 | Benzyl methyl disulfide | C8H10S2 | Organic sulphide |
| 196 | 27.79 | Bicyclo[3.1.0]hexan-2-one, 3,3,6-trimethyl- | C9H14O | Ketone |
| 197 | 27.96 | 2-Phenylacetic acid, 2,2,2-trifluoroethyl ester | C10H9F3O2 | Fluorinated organic compound |
| 198 | 27.99 | Nonanoic acid, 4-biphenyl ester | C21H26O2 | Ester |
| 199 | 28.02 | 1-Tripropylsilyloxymethyladamantane | C20H38OSi | Organosilicon compound |
| 200 | 28.19 | Furan-2-carboxaldehyde, 5-(2,4-difluorophenyl)- | C11H6F2O2 | Fluorinated aromatic compound |
| 201 | 28.30 | Dodecanoic acid, 4-biphenyl ester | C24H32O2 | Ester |
| 202 | 28.38 | Benzoic acid, 3,4-dihydroxy- | C7H6O4 | Aromatic organic compound |
| 203 | 28.38 | 5-Chlorobenzimidazole | C7H5ClN2 | Heterocyclic aromatic compound |
| 204 | 28.43 | 2,5-Piperazinedione, 3-(phenylmethyl)- | C11H12N2O2 | Organic compound |
| 205 | 28.44 | 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)- | C12H22N2O2 | Organic compound |
| 206 | 28.47 | Glycine, N-(m-anisoyl)-, methyl ester | C11H13NO4 | Aromatic organic compound |
| 207 | 28.68 | Benzamide, 3-methyl-N-(2-ethylphenyl)- | C16H17NO | Organic compound |
| 208 | 28.70 | Eicosanoic acid, methyl ester | C21H42O2 | Ester |
| 209 | 28.90 | Tetracosane | C24H50 | Organic compound |
| 210 | 28.91 | 3,5-Dichlorobenzonitrile | C7H3Cl2N | Organic compound |
| 211 | 29.30 | n-Tetracosanol-1 | C24H50O | Organic compound |
| 212 | 29.35 | Octadecanamide | C18H37NO | Organic compound |
| 213 | 29.49 | 6-Hydroxy-3-phenyl-4H-chromen-4-one | C15H10O3 | Organic compound |
| 214 | 29.60 | Cyclo-(l-leucyl-l-phenylalanyl) | C15H20N2O2 | Organic compund |
| 215 | 29.76 | N-Benzyl-2-phenethylamine | C15H17N | Organic compound |
| 216 | 29.77 | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3- | C14H16N2O2 | Organic compound |
| 217 | 29.86 | 1,2-Bis(3-benzyloxy-4-methoxyphenyl)ethylamine | C30H31NO4 | Amine |
| 218 | 30.19 | Diethylene glycol dibenzoate | C18H18O5 | Organic ester  |
| 219 | 30.32 | 7H-Pyrrolo(2,3-d)pyrimidine, 4-chloro- | C6H4ClN3 | Heterocyclic organic compound |
| 220 | 30.58 | 9,10-Anthracenedione, 1,4-dihydroxy-2,3-dimethyl- | C16H12O4 | Aromatic organic compound |
| 221 | 30.72 | Phthalic acid, di(2-propylpentyl) ester | C24H38O4 | Phthalic acid ester |
| 222 | 30.88 | 2-Amino-4-hydroxy-6,7-bis[3-phenylpropyl] pteridine | C24H25N5O | Heterocyclic organic compound |
| 223 | 31.04 | Cyclotetradecane | C14H28 | Cycloalkane |
| 224 | 31.06 | Isophthalic acid, di(2-isopropylphenyl) ester | C26H26O4 | Ester |
| 225 | 31.07 | Hexadecane, 2-methyl- | C17H36 | Organic compound |
| 226 | 31.12 | Phosphoric acid, dihexyl ethyl ester | C14H31O4P | Phosphoric acid ester |
| 227 | 31.79 | Thiamphenicol acetate | C14H17Cl2NO6S | Organic compound |
| 228 | 31.87 | Acetamide, 2-bromo-N-benzyl-N-octyl- | C17H26BrNO | Amide |
| 229 | 32.31 | Chloramphenicol | C11H12Cl2N2O5 | Organic compound |
| 230 | 32.38 | Triacontane | C30H62 | Organic compound |
| 231 | 32.58 | Cyclohexanone, 3-butyl- | C10H18O | Organic compound |
| 232 | 32.84 | Squalene | C30H50 | Organic compound |
| 233 | 32.92 | 2,5-Piperazinedione, 3,6-bis(phenylmethyl)- | C18H18N2O2 | Organic compound |
| 234 | 33.06 | Benzene, 1-bromo-4-iodo- | C6H4BrI | Aromatic organic compound |
| 235 | 33.10 | Butylphosphonic acid, decyl 4-(2-phenylprop-2- | C29H45O3P | Phosphonic acid ester |