*Journal of Applied and Natural Science*
15(3), 1086 - 1094 (2023)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

*Research Article*

# Antimicrobial nature of specific compounds of *Ampelomyces quisqualis* identified from gas chromatography-mass spectrometry (GCMS) analysis and their mycoparasite nature against powdery mildew of grapes

**Ranjan Kumar Jena***
Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai - 625104 (Tamil Nadu), India
**I Yesu Raja**
Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai - 625104 (Tamil Nadu), India
**V Ramamoorthy**
Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Eachangkottai, Thanjavur - 614902 (Tamil Nadu), India
**S Lakshmi Narayanan**
Department of Plant Breeding and Genetics, Tamil Nadu Agricultural University, Madurai - 625104 (Tamil Nadu), India
**R Renuka**
Department of Biotechnology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai - 625104 (Tamil Nadu), India
**Eraivan Arutkani Aiyanathan K**
Department of Plant Pathology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Madurai - 625104 (Tamil Nadu), India
**A Subbiah**
Grape Research Station, Anaimalayanpatty, Tamil Nadu Agricultural University, Theni - 625526 (Tamil Nadu), India
**V Karthik Pandi**
Grape Research Station, Anaimalayanpatty, Tamil Nadu Agricultural University, Theni - 625526 (Tamil Nadu), India

*Corresponding author. E-mail: ranjan.ranjan.jena@gmail.com

***Article Info***
https://doi.org/10.31018/jans.v15i3.4654
Received: April 22, 2023
Revised: August 23, 2023
Accepted: August 27, 2023

***How to Cite***
Jena, R. K. *et al.* (2023). Antimicrobial nature of specific compounds of *Ampelomyces quisqualis* identified from gas chromatography-mass spectrometry (GCMS) analysis and their mycoparasite nature against powdery mildew of grapes. *Journal of Applied and Natural Science*, 15(3), 1086 - 1094. https://doi.org/10.31018/jans.v15i3.4654

**Abstract**
Grapevine powdery mildew is the world's most important plant disease, and *Ampelomyces* frequently fight them. While it does not usually cause plant death, its major infections can result in significant production losses and severely impact wine quality. Fungicides are frequently used to control the disease, which can have long-term adverse effects on the ecosystem. As a result, alternative and environmentally friendly disease management approaches must be developed. The study aimed to reduce costly and toxic fungicide use by using *Ampelomyces*, a natural biofungicide, against various powdery mildew fungi. GC-MS analysis was also used to determine the antagonistic potential and efficacy of volatile organic chemicals produced by several *Ampelomyces* spp. against *Erysiphe necator*, which causes powdery mildew of grapes. The molecular characterization of *A. quisqualis* isolates based on using rDNA ITS region was also carried out and sequenced. GC-MS analysis identified various antimicrobial compounds, such as squalene (4.643%), octadecanoic acid (3.862%), tetradecanoic acid (3.600%), and 9,12-octadecadienoic acid (Z,Z) (1.451%). The least abundant compounds were 2-Hexadecanol, 1-Tricosanol, and 2-propenyl ester, with percentages of 0.485, 0.519, and 0.560, respectively. These bioactive compounds revealed by GC-MS analysis in crude extracts of *A. quisqualis* had a stronger antifungal and antibacterial activity against *E. necator*. As a result, using *A. quisqualis* to control the powdery mildew of grapes significantly reduced pathogen growth and disease incidence.

**Keywords:** *Ampelomyces Quisqualis*, Biocontrol, GC-MS, Powdery mildew, Volatile organic compounds

## INTRODUCTION

Grapevine powdery mildew is a major disease affecting cultivated and wild grapevine species worldwide, resulting in significant yield and economic losses (Gadoury *et al.* 2012; Parag *et al.* 2017). It is caused by *Erysiphe necator* Schwein (previously *Uncinula necator* (Schwein.) Burrill; anamorph Oidium tuckeri Berk.), an obligate biotrophic fungus belonging to ascomycetes, family Erysiphaceae. The epiphytically growing mildew colonies appear as whitish, roughly circular spots that later take on a typical powdery appearance due to abundant asexual conidia production. The pathogen can infect all green tissues of the plant, including leaves, shoots, flowers, and bunches, but flower and berry infections cause the most economic damage (Calonnec *et al.,* 2004, Gadoury *et al.,* 2012). Uncontrolled epidemics of *E. necator* may result in yield losses and a reduction in the quality of the produced wine (Parag *et al*., 2017, Gadoury *et al.* 2012). During the winter, the fungus survives as mycelium in dormant grapevine buds or as chasmothecia, which are fruiting bodies produced by the sexual stage (Gindro *et al.* 2006; Cadle-Davidson *et al.* 2019). Ascosporic infections caused by chasmothecia commonly appear randomly in the vineyard in the spring as scattered whitish and powdery spots on leaves (1 to 3 mm in diameter), primarily on leaves close to the trunk (Ypema *et al.* 2000; Grove *et al.* 2004). Fungicides are used indiscriminately to protect crops from powdery mildew pathogens, resulting in fungicide resistance. Furthermore, fungicides have a negative impact on biodiversity, natural ecosystems, and the residual fungicides problem in food (Fernandes *et al.,* 2020). Physical and biological approaches to powdery mildew management have been proposed to supplement and replace chemical management. In terrestrial environmental conditions, mycoparasites (fungi that parasitize other fungi) are naturally abundant in most powdery mildew infections, particularly in biotrophic interactions (Angeli *et al.* 2012). Numerous mycoparasites have been extensively researched and cost-effectively used as bio-control agents (Keerthana *et al.,* 2022).

*Ampelomyces quisqualis* is a pycnidial hyperparasite on powdery mildew disease that is distributed widely across the world. It is an ecologically and economically important fungus that is highly specific, ecofriendly, and cost-effective in controlling powdery mildew fungi (Liyanage *et al*., 2018). It is one of the first commercially available biocontrol agents for plant diseases, and *A. quisqualis* CNCM I807 is the active ingredient in one of the oldest bio fungicides (Hofstein *et al*., 1996). In nature, the fungus generates conidia in the form of pycnidia, which develop intracellularly in powdery mildew mycelium and restrict mycelial growth, sporulation, and conidial germination (Keerthana *et al.,* 2022). *A. quisqualis* can generate pycnidia *in vitro*, although conidiation on culture media is weak (Philipp and Cruger,1979). On Czapek-Dox agar at 23°C, *A. quisqualis* is a slow-growing fungus with a radial growth rate of 0.5-1 mm d-1(Kiss *et al*., 2004; Angeli *et al*., 2017). It can grow in a variety of liquid media, but mycelial growth and conidia production are highly dependent on medium nutrients, pH, agitation, aeration, and light conditions. In shaken cultures in potato broth, *A. quisqualis* produced the most conidia ($9.7 \times 10^6$) and, interestingly, omitting glucose from the potato dextrose broth caused a significant increase in conidia formation (Keerthana *et al.,* 2022; Angeli *et al*., 2017). Furthermore, 23 °C and 25 °C were the optimal temperatures for conidia germination and for pycnidia formation, respectively. Gu (1998) reported that a strain of *A. quisqualis* isolated from *Podosphaera leucotricha* (powdery mildew of *Malus pumila*) failed to grow in potato broth and that the concentration of conidia produced in vegetable (carrot) broth was higher ($2.7\times10^7$ conidia $mL^{-1}$).

Most *A. quisqualis* research has concentrated on its potential use as a biocontrol agent against powdery mildews of various crops (Angeli *et al*., 2017; Liyanage *et al*., 2018; Keerthana *et al*., 2022). This mycoparasite invades and destroys host cytoplasm, resulting in the death of parasitized powdery mildew cells (Whipps,2001, Keerthana *et al.,* 2022; Kiss *et al*. 2004). *A. quisqualis* intracellular pycnidia are commonly found in powdery mildew hyphae, conidiophores, and immature ascomata (Kiss *et al*. 2004). Conidia in Pycnidia are cylindrical, spindle-shaped, occasionally curved, and two-spotted (Keerthana *et al.,* 2022). Powdery mildew microcyclic conidiogenesis has recently been studied (Kiss *et al*. 2009). When mildew colonies are treated with a suspension of *A. quisqualis* conidia, pycnidia form in microcyclic conidiophores, accelerating *A. quisqualis* asexual reproduction. *A. quisqualis* recognises the presence of host fungi and a water-soluble substance derived from powdery mildew fungi conidia has been shown to stimulate the germination of its conidia *in vitro* (Gu and Ko 1997). Several molecular studies using the internal transcribed spacer (ITS) region of the nuclear ribosomal RNA gene (nrDNA) have revealed significant genetic diversity among *A. quisqualis* strains (Angeli *et al*. 2009a; Damm *et al*. 2017; Cheng *et al*. 2019). The present study chose several *A. quisqualis* strains from various geographical regions of Tamil Nadu that exhibit intracellular pycnidia formation and slow radial growth *in vitro* at room temperature (Keerthana *et al*., 2022). The present study aimed to investigate the antagonistic potential of various *A. quisqualis* strains against powdery mildew of grapes and the volatile organic compounds (VOC) responsible for *E. necator* inhibition using GC-MS gas chromatography spectrometry.

## MATERIALS AND METHODS

### Collection of samples and processing

Grape plant samples infected with powdery mildew were collected from various regions *i.e.* Theni, Dindigul, Coimbatore and Krishnagiri of Tamil Nadu, India. 120 samples were collected from horticultural and agricultural cropping ecosystems in field environmental conditions and stored in refrigerator. Besides that, 25 vineyards were sampled. The vineyards were chosen at random. The vineyards sampling included conventionally and organically managed vines and abandoned (untreated) vineyards. In each vineyard and year, four replicates of 25 leaves were randomly collected at the fifth leaf from the shoot of the plant. The percentage of the infected area on the upper surface of the leaf (whitish, powdery spots) and the number of infected leaves were visually assessed for each replicate. The severity of the disease (percentage of infected leaf area) and the incidence of the disease (percentage of infected leaves) were calculated. *Ampelomyces* pycnidia were isolated using a stereo microscope at a magnification of 20X. The live samples were stored in the

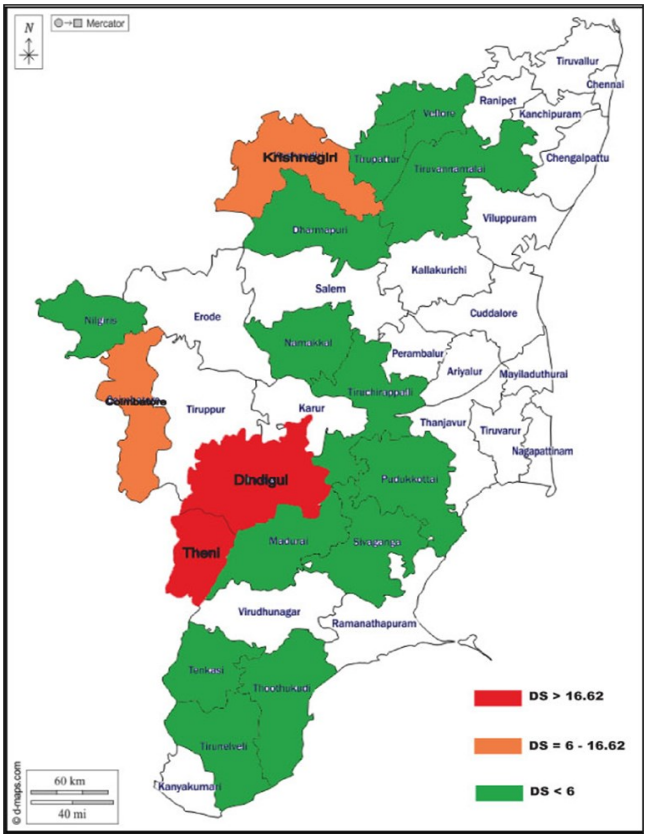


**Fig. 1**. *Showing the surveyed districts (color-filled areas) in the years 2021, 2022 and 2023 (Green color filled districts denotes the less incidence of diseases severity (DS) (less than 6%), Brown color filled districts disease severity ranged from 6% to 16.62% and the red color filled districts having more than 16.62% disease severity.*

growth chamber for one week at a modified temperature of 23±1°C and 83% RH, with a 12:12 dark-to-light ratio (Braun *et al.,*1987).

### Enumeration of Mycoparasite from Powdery Mildew Infection

Microscopic studies were conducted to determine the presence of *Ampelomyces* pycnidia in the mycelium of the Erysiphales species. By placing a spore suspension in a slide and covering it with a cover slip, Pycnidia were observed. The host parasitic relationship was documented using advanced microscopic equipment such as a stereomicroscope, light microscope, phase contrast, and scanning electron microscope. The qualitative and quantitative morphological structures of *A.* pycnidia and pycnidiospores were measured (Angeli, 2009).

### Isolation of *Ampelomyces* sp. using Pycnidia Picking Method

In potato dextrose agar medium, powdery mildew-infected leaves parasitized by *Ampelomyces* sp. were isolated. The pycnidia were examined under a stereo microscope and picked with a sterilised insulin needle before being placed on potato dextrose agar (PDA; Himedia, Mumbai) (Goh, 1999). To avoid contamination, 0.3% streptomycin sulphate or 2% chloramphenicol was added to the culturing medium. Plates were incubated at 20±2 °C while growth and development were monitored. Twenty isolates were collected from various cropping systems in a natural ecosystem.

### Morphological examination of *Ampelomyces sp.*

*Ampelomyces sp*. culture isolates that had been subcultured for 20 days were used for morphological studies using a phase contrast microscope. The radial growth was evaluated in five replicates and the height, texture, and colour of each Petri plate. At 40X magnification, morphological parameters such as pycnidia, pycnidiospores, and the presence of petiolate were measured in each isolate. As described by Keerthana *et al. (*2022), a ten-day-old culture was chopped, scraped with a needle, and mounted on aluminium stubs with double-sided adhesive tape coated with gold palladium to study the characteristics of mycelium under a scanning electron microscope.

### Extraction of genomic DNA

Twenty *Ampelomyces* sp. Isolates were cultured in 100ml Erlenmeyer flasks containing 20ml PDA broth for 10 days before collecting mycelium. The total fungal DNA was extracted from 100 mg of mycelium using the CTAB method (Moller *et al*., 1992). The purified DNA was dissolved in 50µl of Tris 10mM + EDTA 1mM pH 8.0 TE buffer. The integrity of genomic DNA (gDNA)

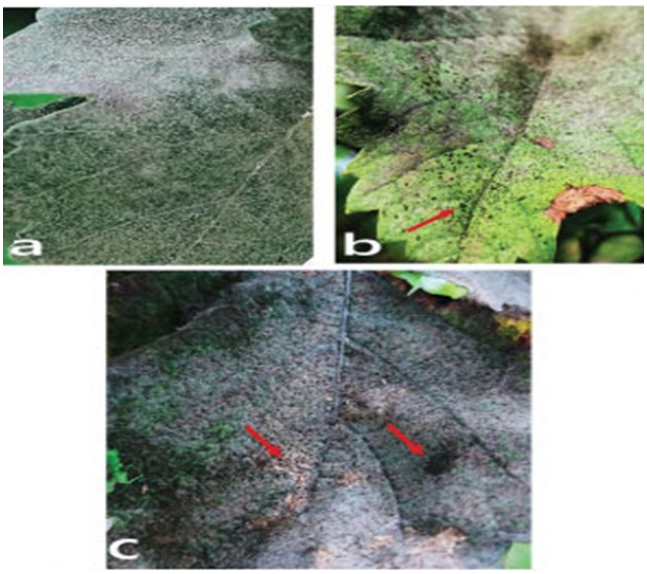


***Fig. 2.*** *Different tages of Ampelomyces quisqualis infection of powdery mildew fungal colonies: (a) healthy colonies of powdery mildew on the surface of grape leaf; (b) powdery mildew colonies infected with Ampelomyces quisqualis (the brown/black color spots are the pycnidia produced by Ampelomyces); (c) powdery mildew colonies totally destroyed by Ampelomyces, 1–2 weeks after infection*

was tested in a 1.5% agarose gel (HiMedia, Mumbai). The NanoDrop1000 spectrophotometer was used to assess the quality and quantity of DNA (Thermo Fisher Scientific NanoDrop 2000c, USA). The DNA concentration was reduced to 50 ng/l and stored at 4°C for future use (Sambrook *et al*., 2006).

**PCR Amplification**

*Ampelomyces* sp. Cultures were identified molecularly using the conserved ribosomal internal transcribed spacer (ITS) region. We amplified the ITS regions between the small nuclear 18S rDNA and the large nuclear 28S rDNA, including 5.8S rDNA, using the universal primer pairs ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*., 1990, Hirata *et al*., 1996). All PCR reactions were performed with the following parameters on a Mastercycler® Nexus X2 PCR cycler (MA, USA): 1) Initial denaturation at 95□ for 10 minutes, followed by 35 cycles at 94□ for 30 seconds, 60□ for 45 seconds, and 72□ for 1 minute, and a final extension at 72□ for 8 minutes on 1.0 % agarose gels. The PCR products (Biotium,Hayward, CA.) were viewed using the Bio-Rad Gel Doc EZ Imaging System.

**Preparation of crude extracts of *Ampelomyces quisqualis***

The crude extracts of the effective *A. quisqualis* were prepared by transferring a 9mm mycelial disc from an actively growing effective *Ampelomyces* isolate (MDU4) into 200ml of potato dextrose broth and incubating it for seven days at 23±1°C. The culture filtrates were obtained by filtering the extracts through Whatmann no.1 filter paper and centrifuging them for 15 minutes at 9000 rpm. The metabolites were extracted from the culture filtrates using the solvent ethyl acetate. The solvent containing VOC was concentrated using a rotary evaporator until the solvent was completely evaporated. The final product was filtered through a 0.4µm bacterial filter after being diluted with 2ml ethyl acetate.

**Gas Chromatography-mass spectrum analysis (GCMS) of crude extracts of *Ampelomyces quisqualis***

Shimadzu Gas chromatography equipped with a Mass detector turbo mass gold containing an Elite -1 (100% Dimethyl Poly Siloxane), 30 m x 0.25 mm ID x one mM df was used to identify various VOC of effective *A. quisqualis*. The following conditions were used: Carrier gas, helium (1 ml/min), oven temperature programme 110 □ (2 min) to 280 □ (9 min), injector temperature (250 □), total GC time (45 min), final output ethyl acetate extracts were injected into the chromatography at 1.0 µl. A computer algorithm was used to identify the major volatile organic compounds present. The analysis was compared to the National Institute of Standards and Technology (NIST) library database and the AMDIS software programme. This GC-MS analysis was performed at Centre of Innovation for Excellence, Agricultural College & Research Institute, Tamil Nadu Agricultural University, Madurai.

## RESULTS AND DISCUSSION

Powdery mildew of grapes caused by *E. necator* is one of India's most widespread diseases, affecting both major and off-season crops. Because crops are grown throughout the year in various geographical regions of India, the disease occurs in epidemic proportions practically every year (Nakova *et al.,* 2017; Shinde *et al.,* 2022). In the present study, the powdery mildew diseased samples were collected from various locations in Tamil Nadu between 2021 and 2023 and their severity is shown in Fig.1, and the presence of mycoparasitic infections were recorded. Mycoparasitic infections were found in 25 distinct locations. However, none was found in the samples collected from other locations. Twenty isolates showed the highest levels of *Ampelomyces* sp. Pycnidia's mycoparasitization. Light microscopic (LM) study of grape powdery mildew colonies demonstrated that specimens taken from Tamil Nadu, India, were infected by *Ampelomyces* spp. even though *Ampelomyces* spp. has been documented as parasitizing grape powdery mildew fungus (Fig.2 a-c).

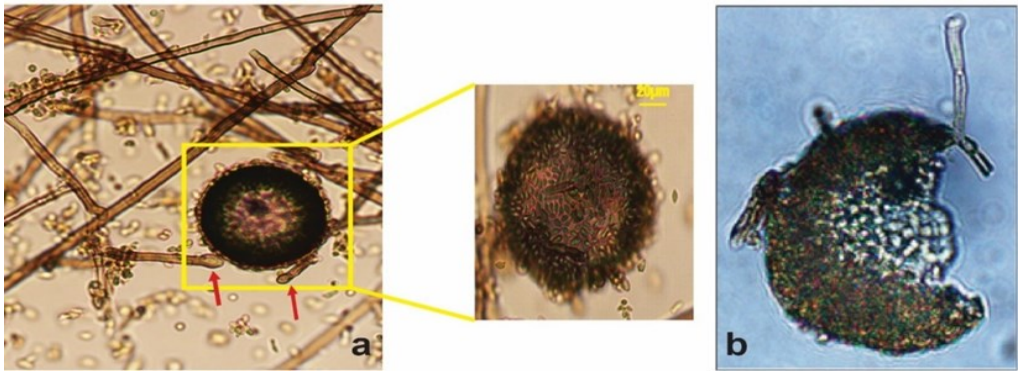


**Fig. 3.** *Microscopic image of Ampelomyces pycnidia (a) pycnidia and pycnidiospores produced in Ampelomyces isolate (red arrow indicates conidia attached to conidiophore); (b) Mature chasmothecia releasing an ascus spores*

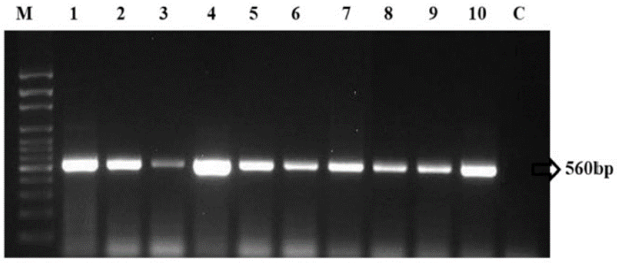


**Fig. 4.** *Molecular identification of Ampelomyces quisqualis species of ITS region*

**Morphological characterization and identification of *Ampelomyces* sp.**

Morphological analysis of *Ampelomyces* from naturally parasitized powdery mildew fungi revealed that the mycoparasite's hyphae were slender, slightly coloured, and located inside the powdery mildew fungi's hyphal cells, conidiophores, and conidia. During the initial stage, mycelia were septate and hyaline. In mature colonies, it changed from greyish-white to brownish-black. Some fully grown culture plates exhibited zonation, with its margins appearing smooth, wavy, or irregular. The mycoparasite's pycnidia varied in shape (round, ovoid, flask, pyriform, globose) and colour (olive green to brown with a reticulate pattern). The pycnidia's size also varied, ranging from 56.24 to 74.20 × 50.23 to 63.81 µm. Pycnidiospores were unicellular, hyaline, and oval in shape, ranging in size from 9.63 to 15.77 × 2.29 to 3.50 µm (Fig.3). The hyphal lengths appeared 48 hours after the injection. Fungal colonies grew slowly and concentrically after a single mature pycnidium was inoculated in the middle of PDA medium.

**Molecular identification**

The ITS region of fungal DNA is extremely valuable for molecular systematics at the species level and within species (for example, identifying geographic races). Variation among individual rDNA repeats can sometimes be noticed within both the ITS and IGS regions due to their higher degree of variation than other regions of rDNA. In the present study, the sequence were shown 97 per cent sequence homology with GenBank sequences with BLASTn analysis. Using Internal Transcribed Spacer (ITS) region, we discovered that the 10 isolates from different areas of Tamil Nadu shared sequence homology with isolates from other regions such as India, China, and Korea. To validate the initial identification and identify the clear taxonomic position, the ITS regions (ITS1 and ITS4) and 5.8S gene area of

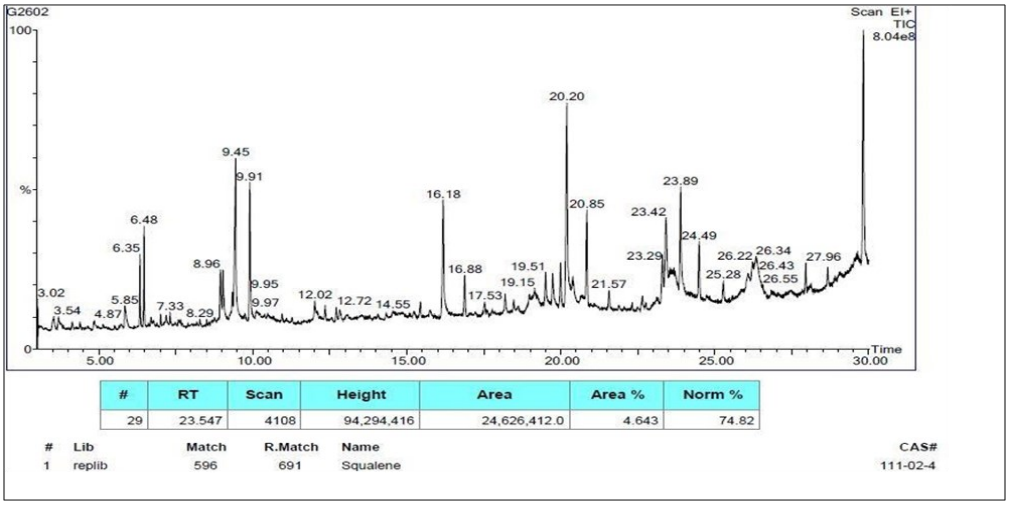


**Fig. 5.** *Total ion chromatogram of secondary metabolites identified from Ampelomyces quisqualis by GC-MS analysis*

18S rDNA were initially amplified with the primers ITS1 and ITS4. All twenty isolates were amplified using 560 base pairs (Keerthana *et al.,* 2022) (Fig. 4). The amplified 18S-rDNA (ITS 1 and ITS 4) region was purified separately and sequenced at National Center for Biotechnology Information (NCBI) using sangar dideoxy sequencing.

**Detection of secondary metabolites by Gas Chromatography-Mass Spectrometry (GC-MS)**

The present study subjected the secondary metabolites produced from Ampelomyces crude extract using methanol solvent to GC-MS analysis. The identity of the compound was confirmed using the NIST Library 2005 and the AMDIS software programme. The crude extracts of the AQS3 isolate contained approximately 40 secondary metabolic compounds (Fig 5). Among the various compounds, squalene has the highest peak of antimicrobial activity at 4.643 %, followed by octadecanoic acid at 3.862 %, tetradecanoic acid at 3.600 %, and 9,12-octadecadienoic acid (Z,Z) at 1.451 %(Fig 6; Fig 7). Similarly, the lowest peak exhibiting compounds, namely 2-Hexadecanol, 1-Tricosanol, and 2-propenyl ester, were detected with 0.485, 0.519, and 0.560 %, respectively (Table 1; Fig 5). The study assumed these VOCs could inhibit the *E. necator*. Similarly, the study of Naznin *et al*. (2014) isolated several VOCs from *Ampelomyces* sp. and these compounds were responsible for reduced disease symptoms and pathogen population.

## Conclusion

This study confirmed the presence of *A. quisqualis* infections in 25 distinct locations. Morphological characterization of *Ampelomyces* revealed slender, colored hyphae, conidiophores and conidia inside the powdery mildew fungi's cell. The mycoparasite's pycnidia showed variations in shape, color and size. Crude extracts of *Ampelomyces* contained forty VOCs. The nine individual volatiles with antifungal activities, namely,

**Table. 1.** *Secondary metabolites identified from crude extracts of Ampelomyces quisqualis through GCMS analysis*

| Sl. No. | Retention time | Peak area per cent | Compound name | Molecular weight (g/mol) | Molecular formula | Biological properties | References |
|---|---|---|---|---|---|---|---|
| 1. | 3.699 | 0.560 | 2-propenyl ester | 86.09 | C4H6O2 | Antifungal | Wang *et al*. (2012) |
| 2. | 5.855 | 0.885 | 4 H-Pyran-4- one, 2,3- dihydro-3,5- dihydroxy 6-methyl- | 144.126 | C6H8O4 | Antibacterial | El-Benawy *et al*. (2020) |
| 3. | 6.350 | 0.929 | Cyclohexano,1-methyl-4 (1-methylethyl) | 156.269 | C10H20O | Antifungal | Wang *et al*. (2010) |
| 4. | 6.480 | 1.348 | Dihydroartemi sinin | 284.352 | C15H24O5 | Antimicrobial | Dai *et al*. (2021) |
| 5. | 8.956 | 1.174 | 3-Decenoic acid | 170.252 | C10H18O2 | Antifungal | Ma *et al*. (1980) |
| 6. | 9.046 | 1.165 | L-Glutamine | 146.146 | C5H10N2O3 | Antitoxin | Wischmeyer *et al.* (2003) |
| 7. | 12.02 | 0.580 | Dodecanoic acid | 200.322 | C12H24O2 | Antifungal | Walters *et al.* (2003) |
| 8. | 16.14 | 3.600 | Tetradecanoic acid | 228.376 | C14H28O2 | Antifungal | Li *et al.* (2012) |
| 9. | 16.84 | 0.883 | 1-Nonadecene | 266.513 | C19H38 | Antifungal | Jayasuriya *et al*. (2003) |
| 10. | 18.199 | 0.543 | Pentadecanoic acid | 242.403 | C15H30O2 | Antifungal | Jenkins *et al*. (2015) |
| 11. | 19.735 | 1.051 | 9-Hexadecenoic acid | 270.457 | C17H34O2 | Antifungal | Oviya *et al*. (2022) |
| 12. | 19.995 | 1.038 | Dibutyl phthalate | 278.348 | C16H22O4 | Antifungal | Czubacka *et al*. (2021) |
| 13. | 21.566 | 0.485 | 2-Hexadecanol | 242.447 | C16H34O | Antifungal | Li *et al.* (2012) |
| 14. | 23.302 | 1.451 | 9,12-Octadecadieno ic acid (Z,Z)- | 280.452 | C18H32O2 | Antifungal | Wang *et al*. (2012) |
| 15. | 23.547 | 4.643 | Squalene | 410.73 | C30H50 | Antimicrobial | Awa *et al*. (2012) |
| 16. | 23.892 | 3.862 | Octadecanoic acid | 284.484 | C18H36O2 | Antifungal | Awa *et al*. (2012) |
| 17. | 27.958 | 0.519 | 1-Tricosanol | 340.636 | C23H48O | Antiviral | Chatterjee *et al*. (2018) |
| 18. | 29.629 | 0.501 | Digitoxin | 764.95 | C41H64O13 | Antifungal | Elbaz *et al*. (2012) |

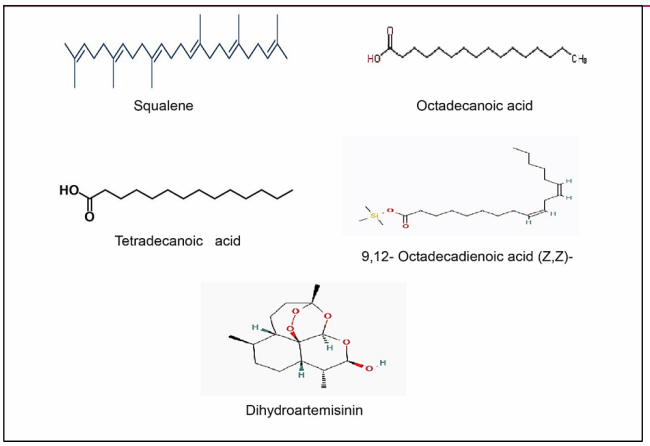


**Fig. 6:** *Chemical structure of important antifungal compounds produced by Ampelomyces quisqualis*

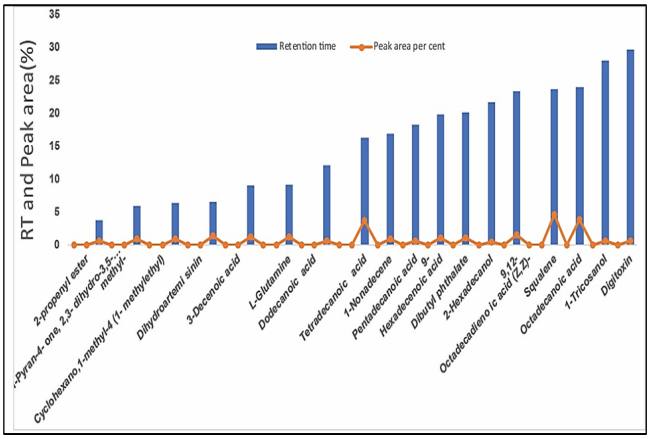


**Fig. 7** *RT and peak area of major antifungal compounds*

squalene, octadecanoic acid, tetradecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, Dihydroartemi sinin, 3-Decenoic acid, L-Glutamine, 9-Hexadecenoic acid and Dibutyl phthalate were key inhibitory VOCs and they are important for their broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria, as well as *E. necator* . Hence, these findings provide valuable insights for further research on the potential use of *A. quisqualis* as a biocontrol agent against powdery mildew disease in grapes and other crops.

**Conflict of interest**

The authors declare that they have no conflict of interest.

## REFERENCES


1. Angeli, D., Pellegrini, E. & Pertot, I. (2009). Occurrence of *Erysiphe necator* chasmothecia and their natural parasitism by *Ampelomyces quisqualis*. *Phytopathology*.99(6), 704-10. https://doi: 10.1094/PHYTO-99-6-0704
2. Angeli, D., Pellegrini, E., Micheli, S., Ress, D., Maurhofer, M. & Pertot, I. (2009a). Molecular characterization of *Ampelomyces* spp. strains from different hosts and geographic origins and evaluation of their potential to control powdery mildew of cucumber. *IOBC/WPRS Bulletin*, 43, 40–44. http://www.iobc-wprs.org/pub/bulletin...
3. Angeli, D., Puopolo, G., Maurhofer, M., Gessler, C. & Pertot, I. (2012). Is themycoparasitic activity of *Ampelomyces quisqualis* biocontrol strains related to phylogeny and hydrolytic enzyme production. *Biol. Control.*, 63: 348-358.
4. Angeli, D., Saharan, K., Segarra, G., Sicher, C., & Pertot, I. (2017). Production of *Ampelomyces quisqualis* conidia in submerged fermentation and improvements in the formulation for increased shelf-life. *Crop Protection*, 97, 135-144. doi.org/10.1016/j.cropro.2016.11.012
5. Awa, E. P., Ibrahim, S., & Ameh, D. A. (2012). GC/MS analysis and antimicrobial activity of diethyl ether fraction of methanolic extract from the stem bark of Annona senegalensis Pers. *International Journal of Pharmaceutical Sciences and Research*, *3*(11), 4213.
6. Braun U. (1987). A monograph of the *Erysiphales* (powdery mildews). *Beihefte zur Nova Hedwigia*. (89).
7. Cadle-Davidson., Jason, L., Dani, M., Surya, S. (2019). Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Molecular Plant Pathology*, 4(20), 421-438.
8. Calonnec, A., Cartolaro, P., Poupot, C., Dubourdieu, D., Darriet, P. (2004). Effects of *Uncinula necator* on the yield and quality of grapes (*Vitis vinifera*) and wine. *Plant Pathol*, 53, 434–445. doi.org/10.1111/j.0032-0862.2004.01016.x
9. Chatterjee, S., Karmakar, A., Azmi, S. A., & Barik, A. (2018). Antibacterial activity of long-chain primary alcohols from Solena amplexicaulis leaves. *In Proceedings of the Zoological Society*, Vol. 71, 313-319. Springer India.
10. Cheng, X., Xue, X., Ma, S., Sun, J., Zhang, G., & Li, Y. (2019). Genetic diversity and population structure of *Ampelomyces quisqualis* isolated from different host plants and geographical locations in China. *European Journal of Plant Pathology*, 153(4), 1163-1173.
11. Czubacka, E., Czerczak, S., & Kupczewska-Dobecka, M. (2021). The overview of current evidence on the reproductive toxicity of dibutyl phthalate. *International Journal of Occupational Medicine and Environmental Health*, *34*(1).
12. Dai, X., Zhang, X., Chen, W., Chen, Y., Zhang, Q., Mo, S., & Lu, J. (2021). Dihydroartemisinin: a potential natural anticancer drug. *International Journal of Biological Sciences*, *17*(2), 603.

13. Damm, U., Cannon, P. F., Woudenberg, J. H., Johnston, P. R., Weir, B. S., Tan, Y. P., & Crous, P. W. (2017). The *Colletotrichum* boninense species complex. *Studies in Mycology*, 87, 1-41.
14. Elbaz, H. A., Stueckle, T. A., Tse, W., Rojanasakul, Y., & Dinu, C. Z. (2012). Digitoxin and its analogs as novel cancer therapeutics. *Experimental hematology & oncology*, *1*(1), 1-10.
15. El-Benawy, N. M., Abdel-Fattah, G. M., Ghoneem, K. M., & Shabana, Y. M. (2020). Antimicrobial activities of *Trichoderma atroviride* against common bean seed-borne *Macrophomina phaseolina* and *Rhizoctonia solani*. *Egyptian Journal of Basic and Applied Sciences*, *7*(1), 267-280.
16. Fernandes, G. W., Arantes-Garcia, L., Barbosa, M., Barbosa, N.P., Batista, E.K., Beiroz, W., Resende, F.M., Abrahao, A., Almada, E.D., Alves, E., Alves, N.J. (2020). Biodiversity and ecosystem services in the Campo Rupestre: A road map for the sustainability of the hottest Brazilian biodiversity hotspot. *Perspectives in Ecology and Conservation*, 18(4), 213-22. doi.org/10.1016/j.pecon.2020.10.004
17. Gadoury, D.M., Cadle-Davidson, L., Wilcox, W.F., Dry, I.B., Seem, R.C., Milgroom, M.G. (2012). Grapevine powdery mildew (*Erysiphe necator*): A fascinating system for the study of the biology, *ecology and epidemiology of an obligate biotroph*. *Mol. Plant Patho*logy, 13, 1–16. DOI: 10.1111/j.1364-3703.2011.00728.x
18. Gindro, Harikrishnan, R., Rio, L. E. (2006). Identification and characterization of *Erysiphe necator* strains resistant to strobilurins. *Plant Disease*, 7(90), 975-980.
19. Goh, T.K. (1999). Single spore isolation using a hand-made glass needle*. Fungal Diver.*, 2: 47-63.
20. Grove, G. G. (2004). Perennation of *Uncinula necator* in vineyards of Eastern Washington. *Plant Dis*. 88, 242-247. DOI: 10.1094/PDIS.2004.88.3.242
21. Gu, Y. H., & Ko, W. H. (1997). Water agarose medium for studying factors affecting germination of conidia of *Ampelomyces quisqualis*. *Mycological Research*, 101, 422–424. doi.org/10.1017/S09537562 9600295X
22. Gu, Y. H. (1998). Liquid culture of *Ampelomyces quisqualis*, a mycoparasite for biological control of powdery mildews. *Japanese Journal of Phytopathology*, *64*(5), 458-461.
23. Hirata, T., & Takamatsu, S. (1996). Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. *Mycoscience*, *37*(3), 283 -288. doi.org/10.1007/BF02461299
24. Hofstein, R., Daoust, R.A., Aeschlimann, J.P., (1996). Constrains to the development of biofungicides: the example of ‘‘AQ10’’, a new product for controlling powdery mildews. *Entomophaga,* 41, 455-460. doi:10.1094/ PHYTO-99-6-0704
25. Jayasuriya, K. E., Wijesundera, R. L. C., & Deraniyagala, S. A. (2003). Isolation of anti□fungal phenolic compounds from petioles of two *Hevea brasiliensis* (rubber) genotypes and their effect on *Phytophthora meadii*. *Annals of applied biology*, *142*(1), 63-69.
26. Jenkins, B., West, J. A., & Koulman, A. (2015). A review of odd-chain fatty acid metabolism and the role of pentadecanoic acid (C15: 0) and heptadecanoic acid (C17: 0) in health and disease. *Molecules*, *20*(2), 2425-2444.
27. Keerthana, S., Sendhilvel, V., Raguchander, T., Varanavasiappan, S., & Swarnapriya, R. (2022). Diversity of Powdery Mildew Mycoparasite *Ampelomyces quisqualis* under Natural Ecosystem and Its Molecular Characterization. *International Journal of Plant & Soil Science*, 34(9), 48–59. doi.org/10.9734/ijpss/2022/v34i930913
28. Kiss, L., Russell, J.C., Szentivanyi, O., Xu, X., Jeffries, P. (2004). Biology and biocontrol potential of *Ampelomyces* mycoparasites, natural antagonists of powdery mildew fungi. *Biocontrol Science and Technology*, 14(7), 635-51. doi.org/10.1080/09583 150410001683600
29. Kiss, L., Pintye, A., Zseli, G., Jankovics, T., Szentivanyi, O., Hafez, Y. M., (2009). Microcyclic conidiogenesis in powdery mildews and its association with intracellular parasitism by *Ampelomyces*. *European Journal of Plant Pathology*, 126, 445–451.
30. Li, L., Wang, Q., Yang, Y., Wu, G., Xin, X., & Aisa, H. A. (2012). Chemical components and antidiabetic activity of essential oils obtained by hydrodistillation and three solvent extraction methods from *Carthamus tinctorius* L. *Acta Chromatographica*, *24*(4), 653-665.
31. Liyanage KK, Khan S, Brooks S, Mortimer PE, Karunarathna SC, Xu J and Hyde KD (2018) Morpho-Molecular Characterization of Two *Ampelomyces* spp. (Pleosporales) Strains Mycoparasites of Powdery Mildew of *Hevea brasiliensis*. *Front. Microbiol*. 9, 12. doi: 10.3389/fmicb.2018.00012
32. Ma, M., Hummel, H. E., & Burkholder, W. E. (1980). Estimation of single furniture carpet beetle (Anthrenus flavipes LeConte) sex pheromone release by dose-response curve and chromatographic analysis of pentafluorobenzyl derivative of (Z)-3-decenoic acid. *Journal of Chemical Ecology*, *6*, 597-607.
33. Moller E.M., Bahnweg G., Sandermann H and Geiger H.H. (1992). A simple and efficient protocol for isolation of high molecular weight DNA from filamentous fungi, fruit bodies, and infected plant tissues. *Nucleic Acids Research*, 22(20), 6115-6116

34. Nakova, M. B., Nakov, B. K., & Tityanov, M. (2017). Grapevine powdery mildew (*Uncinula necator* (Schw.) Burr.)–a permanent issue concerning the health status of grapes cenosis in Bulgaria. In *BIO Web of Conferences*, 9, 01021. *EDP Sciences.* doi.org/10.1051/bioconf/20170901021
35. Naznin, H.A., Kiyohara, D., Kimura, M., Miyazawa, M., Shimizu, M. & Hyakumachi, M. (2014). Systemic resistance induced by volatile organic compounds emitted by plant growth-promoting fungi in *Arabidopsis thaliana*. *PLoS One*, 9, 1-10. DOI: 10.1371/journal.pone.0086882
36. Oviya, R. *et al.* (2022). Antagonistic potential of *Trichoderma hamatum* against *Alternaria porri* causing purple blotch disease of onion through Gas chromatography-mass spectrometry (GCMS) analysis. *Journal of Applied and Natural Science*, 14(3), 1031- 1038. doi.org/10.31018/jans.v14i3.3814
37. Parag, D., Gawande, P.V., & Mate, G.D. (2017). Management of powdery mildew of okra caused by *Erysiphe cichoracearum*. *Int. J. Curr. Microbiol. App. Sci*, 6(8), 3189 - 3198.
38. Philipp, W.D. and Cruger, G. (1979). Parasitismus von *Ampelomyces quisqualis* auf Echten Mehltaupilzen an Gurken und anderen Gemusearten. *Zeitschrift fur Pflanzenkrankheiten und flanzenschutz*, 86, 129-142.
39. Sambrook, J., Russell, D.W. (2006). Fragmentation of DNA by sonication. *Cold spring harbor protocols*, (4), 4538. DOI: 10.1101/pdb.prot4538
40. Shinde, K. R., Narute, T. K., Sonawane, R. B., & Dalvi, S. G. (2022). Studying the incidence and distribution of the grape powdery mildew disease in Maharashtra state's primary grape-growing regions. *The Pharma Innovation Journal*, 11(9), 723-726
41. Walters, D. R., Walker, R. L., & Walker, K. C. (2003). Lauric acid exhibits antifungal activity against plant pathogenic fungi. *Journal of Phytopathology*, *151*(4), 228-230.
42. Wang, H., Wang, J., & Liu, J. (2010). Determination of flavour compounds in pig milk by simultaneous distillation extraction or solid phase microextraction combined with gas chromatography mass spectrometry. *Chinese Journal of Animal Science*, *46* (17), 62-66.
43. Wang, Y., Chang, L., Zhao, X., Meng, X., & Liu, Y. (2012). Gas chromatography-mass spectrometry analysis on compounds in volatile oils extracted from Yuan Zhi (Radix Polygalae) and Shi Chang Pu (Acorus Tatarinowii) by supercritical CO2. *Journal of Traditional Chinese Medicine*, *32*(3), 459-464.
44. Whipps, J. M. (2001). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, 52(487), 487-511.
45. White, T.J., Bruns, T., Lee, S.J., Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: *A guide to methods and applications*, 18(1), 315-22. doi.org/10.1016/B978-0-12-372180-8.50042-1
46. Wischmeyer, P. E. (2003). Clinical applications of L □glutamine: past, present, and future. *Nutrition in clinical Practice*, *18*(5), 377-385.
47. Ypema, H. L., & Gubler, W. D. (2000). The distribution of early season grapevine shoots infected by *Uncinula necator* from year to year: A case study in two California vineyards. *Am. J. Enol. Vitic*, 51, 1-6. DOI: 10.5344/ajev.2000.51.1.1