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Bioactive volatile organic compounds from diverse *Metarhizium anisopliae* isolates: GC–MS/MS analysis and insecticidal potential against *Spodoptera litura*

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ABSTRACT

This study delves into exploring the insecticidal potential of various isolates of Metarhizium anisopliae by documenting and analyzing their volatile secondary metabolites. The primary objective was to identify isolates showcasing promising biocontrol properties, thereby emphasizing sustainable alternatives to synthetic pesticides. Twelve isolates of M. anisopliae were subjected to examination, with GC-MS/MS analysis utilized for the identification of volatile organic compounds. Laboratory bioassays were conducted against Spodoptera litura, with recorded mortality rates serving as indicators of effectiveness for each isolate. A central focus of investigation was the correlation between the number of volatile metabolites and insecticidal activity. Results indicated that among the isolates, UASR BC-Ma38 exhibited the highest percentage of mortality (98.89 per cent) in bioassays against S. litura, which correlated with the presence of 28 volatile metabolites, primarily demonstrating insecticidal activity. Conversely, ICAR-NBAIR-Ma14 displayed the lowest mortality percentage (45.56 per cent), along with 14 identified metabolites. In conclusion, the study underscores the efficacy of certain M. anisopliae isolates, particularly UASR BC-Ma38, as potential biological control agents against S. litura. Moreover, the correlation between the abundance of volatile metabolites and insecticidal activity highlights the significance of these compounds in augmenting the virulence of isolates, thereby contributing to the development of sustainable pest management strategies leveraging entomopathogenic fungi.

Keywords: *Bioactive volatile organic compounds, Metarhizium anisopliae, GS-MS/MS analysis, Insecticidal potential, Spodoptera litura, Secondary metabolites.*

Introduction

Spodoptera litura (Fab.), commonly known as the tobacco caterpillar or cotton leafworm, is a nocturnal moth belonging to the family Noctuidae and order Lepidoptera. This polyphagous pest poses a serious threat to Agriculture in Asia, Oceania and the Indian subcontinent, infesting a staggering 87 species of host plants. First described by Johan Christian Fabricius in 1775, *S. litura* larvae exhibit vigorous feeding patterns, often resulting in complete destruction of leaves[64].The economic impact of this pest is devastating, with reported yield losses of 71 per cent in groundnut and 23-50 per cent in tobacco crops in southern India alone. With strong reproductive abilities, ecological adaptation, severe generation overlap and irregular outbreaks, *S. litura* poses a continuous and formidable threat to host crops[1]. As a result, extensive efforts have been undertaken to control this pest and mitigate its impact on agricultural production[44].

The role of *S. litura* as a limiting factor in agricultural productivity has led to the development of various methods for its management. One commonly employed approach is the use of insecticides, which initially appealed to growers due to their easy availability, ability to quickly suppress pest populations and potential for increasing productivity[10]. However, the widespread use of chemical insecticides, including pyrethroids, has resulted in the development of resistance in *S. litura*[3]. This resistance poses a significant challenge in effectively controlling the pest. Moreover, the indiscriminate use of pesticides has had detrimental effects on the environment, poisoning various components of the biosphere. These effects include the resurgence of pests and the reduction of natural enemies in agroecosystems, which, in turn, contribute to the rapid rebound of the pest.

In the contemporary context, there is a growing interest in the exploration of bioactive compounds sourced from microorganisms as alternatives to chemical insecticides[53]. These biopesticides offer a range of advantages, including biodegradability, precise targeting, eco-friendliness and their potential to address the issue of insecticide resistance in pest populations[28,39]. Numerous studies have investigated the control potential of entomopathogenic fungi and their metabolites against a diverse array of insect pests[19,43]. Secondary metabolites derived from various sources, including *Chrysosporium*[48], *Metarhizium* and *Beauveria*[35,12], *Culicinomyces*[46], *Verticillium*[48] and *Piper*[41], have been comprehensively evaluated for their potential as insecticidal agents against a wide spectrum of insect pests[38]. Furthermore, these metabolites exhibit a diverse range of activities, encompassing antibacterial, antifungal, anticancer, antioxidant, feeding deterrent, insect repellent and

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antiviral properties, thereby positioning them as promising candidates for the development of novel bioactive agents[23,37,55]. These findings highlight the considerable potential of metabolites originating from entomopathogenic fungi in acting as effective insecticidal agents within pest management programs. Consequently, in this study, we present our results, evaluating the insecticidal activity of metabolites derived from 12 isolates of *M. anisopliae* against *S. litura.*

Materials and Methods

Research Site

The research was carried out at the ICAR-AICRP on Biocontrol, University of Agricultural Sciences, Raichur, Karnataka, over a span of five months, from January 2024 to May 2024.

Source of *M. anisopliae* isolates

Four isolates of *M. anisopliae* were sourced from ICAR-NBAIR, including ICAR-NBAIR-Ma4 (isolated from *Ploceaderus ferrugineus*), ICAR-NBAIR-Ma14 (isolated from the rhizosphere soil of chilli), ICAR-NBAIR-Ma16 (isolated from *Plutella xylostella*) and ICAR-NBAIR-Ma35 (isolated from soil). In addition, eight isolates were obtained from ICAR-AICRP on Biocontrol, University of Agricultural Sciences, Raichur, comprising UASR BC-Ma2, UASR BC-Ma7, UASR BC-Ma26, UASR BC-Ma27, UASR BC-Ma31, UASR BC-Ma35, UASR BC-Ma38, and UASR BC-Ma60 (isolated from agricultural soils in North-Eastern Karnataka). All these isolates were sub-cultured on Sabouraud's Dextrose Yeast Extract Agar Medium (SDYA), incubated for 14 days at 25 ± 2 °C, and subsequently sub-cultured onto SDYA slants. These subcultures were preserved at -20 °C for use in experimental studies.

Fungal inoculum preparation

The 12 isolates of *M. anisopliae* were characterized for their virulence in laboratory bioassays. Spore suspension was prepared by adding one gram of 14 day-old fungal culture grown on SDYA to nine mL of sterile distilled water containing 0.01 per cent Tween 80. The suspension was vortexed for 5 min and filtrated through three layers of muslin cloth to get hyphal-free spore suspension and the spore concentration was adjusted to various concentrations such as 1×10^2, 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 spores per mL using Neubauer's improved haemocytometer. Conidial viability was determined by plating preparations onto SDYA medium and examining 25 colonies per plate for each of three replicates to check the colony-forming units (CFUs) of the fungal isolate. An aliquot of the suspension was taken to check the viability of the conidia[52].

Bioassay

A bioassay test was carried out in a completely randomized design (CRD) under laboratory conditions to determine the mortality. Third instar larvae of *S. litura* were dipped for 10 seconds in conidial suspension of the concentrations ranging from 1×10^2 to 1×10^9 conidia per mL to ensure contact with conidia of the fungus. The larvae were selected after two days of moulting, since the newly formed integument was highly susceptible to conidial germination and infection. For each concentration, 30 larvae of uniform age were treated in three replications. The untreated control was maintained simultaneously by dipping in only 0.02 per cent Tween 80 in sterile distilled water. After air drying, the treated larvae were carefully transferred and reared in the containers by providing fresh food daily.

The larval mortality was recorded at 4, 6, 8, 10, 12, and 14 days after treatment (DAT). Dead and moribund larvae were removed in separate containers and placed in a humid chamber to allow the growth of the fungus. The larvae died within 24 h post inoculation, with no evidence of mycosis were assumed to be dead due to an unknown cause and such larvae were not considered in analysis[49]. Finally, per cent mortality of *S. litura* larvae for each of the three replicates was calculated[45]. This study was conducted at room temperatures of 25 ± 2 °C and RH (70 ± 10 %). The mortality data were corrected by Abbott's formula[2] and then subjected to 'arcsine' transformation[18].

$$\text{Percent mortality} = \frac{\text{No. of larvae dead due to infection}}{\text{Total number of larvae treated}} \times 100$$

Extraction and Identification of Volatile Organic Compounds

Detection of bioactive compounds present in 12 isolates of *M. anisopliae* was identified by using Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS). The 12 isolates of *M. anisopliae* were grown on potato dextrose broth (PDB) in 500 mL conical flasks containing 250 mL of medium at 28 ± 1 °C for 14 days. After incubation for 14 days, the fully grown fungal flasks were kept on a rotary shaker at 28 ± 1 °C and 150-200 rpm for 2-3 days. The culture was filtered under reduced pressure through two layers of Kimwipes and then through Whatman's No. 1 filter paper. The culture filtrate was extracted twice for 12 hours with an equal volume of ethyl acetate. The organic layer of solvent, which may contain bioactive compounds, was collected through a separating funnel into conical flasks. The solvent was evaporated in vacuo, and the crude extracts were dissolved in 1 mL ethyl acetate for GC-MS/MS analysis. GC-MS/MS parameters were performed by using an Agilent 7890B GC with 7000C MS system, used for identification and quantification with oven temperature of 75 °C for 1 min and 30 °C/min to 300 °C for 2 min; inlet and transfer line temperature is programmed at 280 °C and 290 °C, respectively. The flow rate of helium gas is 1.0 mL/min. 1 µL samples were injected under split of 3:1. The ionization mass spectroscopic analysis was done with 70eV. Interpretation of mass spectrum GC-MS/MS analysis was done by matching a list of known compounds' spectra with Agilent's GC-MS/MS Mass Hunter, NIST MS Library, and NIST's Automated Mass Spectral Deconvolution and Identification Software.

Statistical Analysis

The data was per cent arcsine transformed, and before being subject to analysis of variance (ANOVA) to determine the significance of intra specific differences. Significance was accepted at $p<0.01$ for a completely randomized block design with three replicates. Per cent mycosis was calculated as the proportion of treated dead larvae that showed mycosis and arcsine transformed. The mean of per cent arcsine mortality and mycosis data (from all replicates) in each treatment was back transformed to normalize the data[18].

Results

Bioassay

The efficacy of native isolates against *S. litura* revealed that the fungal isolate UASR BC-Ma38 exhibited the highest mortality of 98.89 per cent and followed by UASR BC-Ma35, ICAR-NBAIR-Ma4 (Standard check), and UASR BC-Ma60, which recorded 93.33, 90.00, and 86.67 per cent mortality respectively. On the other hand, the fungal isolate ICAR-NBAIR-Ma14 demonstrated the least mortality of 45.56 per cent, followed by UASR BC-Ma26, UASR BC-Ma27, and UASR BC-Ma31, which recorded 56.67, 57.78 and 66.67 per cent, respectively on the fourteenth

day after treatment (Table 1). Notably, all isolates demonstrated an increase in mortality with higher concentrations.

Volatile organic compounds

An investigation was conducted to identify bioactive volatile secondary metabolites produced by 12 isolates of *M. anisopliae*. The ethyl acetate crude extract from these *M. anisopliae* isolates underwent Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) analysis to accurately identify bioactive compounds (Fig. 1). In total, 78 compounds were identified across the 12 isolates of *M. anisopliae*, with specific compound counts as follows: 14 compounds in ICAR-NBAIR-Ma14, 20 compounds in ICAR-NBAIR-Ma4, 21 compounds in UASR BC-Ma27, 22 compounds in UASR BC-Ma60, 24 compounds in ICAR-NBAIR-Ma16, ICAR-NBAIR-Ma35, UASR BC-Ma26, UASR BC-Ma2, UASR BC-Ma7, UASR BC-Ma31, UASR BC-Ma35, and 28 compounds in UASR BC-Ma38 (Table 2 and Fig. 1). Notably, three compounds, namely Diethyl pyridine-3,4-dicarboxylate, Methanesulphonamide, N-(2-pentyl)-N-ethyl- and N-[3-[N-Aziridyl]propyl]-3-dimethylaminopropylamine, were found consistently in all isolates, indicating their presence across the samples. Another compound, Ethanone, 1-(3-pyridinyl), was present in all isolates except UASR BC-Ma26, while S-Allyl-L-cysteine, N-(n-butyl)-, n-butyl ester was identified in all isolates except UASR BC-Ma2. Additionally, several other compounds showed varying degrees of presence among the isolates. For instance, Acetamide, N-butyl-N-propyl- was present in 10 isolates but absent in ICAR-NBAIR-Ma14 and UASR BC-Ma35. Tris (aziridinomethyl) hydrazine was present in 10 isolates but absent in UASR BC-Ma7 and UASR BC-Ma35. Furthermore, compounds such as 9,10-Anthracenedione, 1-amino-, Benzonitrile, 4-ethenyl-, Acetamide, 2-chloro-N-propyl-N-tetradecyl-, Beryllium, bis(2,4-pentanedionato-O,_O')-, (T-4)-, 9,12-Octadecadienoic Acid (Z,_Z)-, 1,3-Propanediamine, N,_N-diethyl-N'-methyl- and Acetamide, N-methyl-N-hexadecyl- showed presence in specific isolates while being absent in others. As these compounds were found in at least 6 isolates, they are considered major bioactive volatile secondary metabolites of *M. anisopliae*. These findings highlight the common occurrence of certain compounds across the majority of isolates and the variability in the presence of a few compounds among the isolates.

Discussion

The highest mortality rate was observed at a concentration of 1×10^9 spores per mL, demonstrating greater effectiveness compared to other concentrations. In *S. frugiperda*, a mortality rate exceeding 80 percent was recorded when exposed to six isolates of *M. rileyi* at a concentration of 1×10^{10} spores per mL[29]. Isolates of *M. anisopliae* were used to target *Psylloides chrysocephala* larvae in an effort to identify a stable and highly virulent strain suitable for laboratory conditions[15]. Additionally, various isolates of *B. bassiana* were tested for efficacy against the blister beetle *Lytta nuttali*[34]. In *S. litura*, an 86.50 percent mortality rate was observed after a 10-day exposure to the highest concentration[26]. The *M. anisopliae* isolates CLO 53 and CLO 54 exhibited the highest mortality rate of 90 percent after 10 days of post-inoculation, outperforming other isolates[7]. Similarly, the ICIPE 78 and MA/GPK isolates of *M. anisopliae* demonstrated high virulence, with mortality rates between 77.9 and 82.6 percent, surpassing other isolates[57]. Consistent with these findings, the highest larval mortality in *S. litura* and *H. armigera* was recorded at a concentration of 1×10^9 spores per mL of *M. rileyi*[31]. Furthermore, in *H. armigera*, mortality rates of 78.89 percent at 1×10^8 spores per mL and 85.92 percent at 1×10^{10} spores per mL were documented[20].

The data indicated a steady and cumulative rise in mortality as spore concentration increased. The highest mortality rates were consistently recorded at the elevated concentration of 1×10^9 spores per mL throughout the observation period. A strong positive correlation was observed between the number of infective spores and the mortality induced by mycosis[13]. The vulnerability of the target insect to fungal infection largely depends on spore concentration, with mortality rates rising over time[59]. Similarly, research on the efficacy of various entomopathogenic fungal strains against different aphid species under controlled laboratory conditions confirmed that higher spore concentrations resulted in greater aphid mortality[5]. Comparable trends were noted in the present study with third instar larvae of *S. litura*. Consistent with these findings, mortality rates were initially lower during the first two days but increased significantly thereafter[4]. Collectively, these studies highlight the concentration-dependent and time-sensitive impact of entomopathogenic fungi on insect mortality.

In recent times, there has been a significant surge in interest regarding the utilization of biologically derived pesticides as a viable substitute for synthetic chemicals[6,42,14,53]. Among these, metabolites derived from entomopathogenic fungi hold particular promise due to their numerous advantages over synthetic pesticides. Notably, they are effective in targeting mosquitoes at various stages of their life cycle while exhibiting lower toxicity towards non-target organisms. Furthermore, these metabolites demonstrate remarkable stability at both cold and room temperatures, making them viable for extended storage periods of several months[54,8]. The compound 1-Octen-3-ol, identified in UASR BC-Ma31, UASR BC-Ma38 and UASR BC-Ma60, has been recognized for its insecticidal properties, supporting previous findings that highlight its potential as a natural insecticide for pest management[21]. This compound is commonly found in fungi and various plant species, contributing to the volatile components of essential plant oils[27,16,47,60]. Despite their widespread occurrence, the ecological roles of such compounds remain largely unexplored[58]. These volatile organic compounds (VOCs), produced by *Metarhizium* and other entomopathogenic fungi, play a role in either attracting or eliminating insect pests[58,11]. Recent studies have also demonstrated that 1-Octen-3-ol exhibits both mollusc-repellent and molluscicidal properties[25].

The secondary metabolites identified from *M. anisopliae* in the present study align closely with findings from earlier research, which identified several significant bioactive compounds with insecticidal potential. Notably, 9,12-Octadecadienoic Acid (Z,Z)- was consistently found across multiple isolates, including ICAR-NBAIR-Ma4, ICAR-NBAIR-Ma35, UASR BC-Ma2, UASR BC-Ma7, UASR BC-Ma35, and UASR BC-Ma38 [30,53]. Similarly, 1,2-Benzenedicarboxylic Acid, Diisooctyl Ester was specifically detected in ICAR-NBAIR-Ma16, while 9-Eicosyne was predominantly present in UASR BC-Ma38.

Quinoline obtained in the isolate UASR BC-Ma31 was previously extracted from *Ruta chalepensis* leaves, reported to exhibit strong insecticidal activity against *Sitophilus oryzae*, with an LD_{50} of 0.063 mg/cm^2 [24]. Compound ethanone, 1-(3-pyridinyl), commonly known as 3-acetylpyridine, was found in all isolates studied, except UASR BC-Ma26. This compound functions as a contact insecticide, interfering with the nervous system and leading to paralysis and death in various pests.

Its efficacy has been demonstrated against cockroaches, ants, mosquitoes, and the brown planthopper, often outperforming conventional insecticides like DDT and pyrethroids. Additionally, 3-acetylpyridine is considered safe for humans and mammals, making it a promising agent for sustainable agricultural use due to its mechanism of action, disruption of nerve impulse transmission [17,50].

Another bioactive metabolite, 5-Aziridinopentanol, was identified only in two isolates, ICAR-NBAIR-Ma4 and UASR BC-Ma60. This compound is known to induce oxidative stress and promote cell death in multiple insect species, including mosquitoes, cockroaches, and locusts [56]. Likewise, Acetamide, N-butyl-N-propyl-, was widely present across the majority of isolates, with the exception of ICAR-NBAIR-Ma14 and UASR BC-Ma35. This compound exhibits a range of insecticidal actions, such as ovicidal, repellent, and lethal effects, signifying its broad-spectrum utility in pest management programs [61]. The compound 3,11-Diazatricyclo[7.3.1.0(3,8)]trideca-5,7-dien-4-one, 11-(2-hydroxyethyl)- was detected exclusively in UASR BC-Ma7 and UASR BC-Ma31. Previously isolated from *Anabasis articulata* stems, this molecule has demonstrated both antioxidant and antimicrobial properties [9]. Additionally, 9,10-Anthracenedione, 1-amino- was identified in eight different isolates, while 1,2-Benzenedicarboxylic Acid, Diisooctyl Ester, reaffirming its earlier mention, was uniquely found in ICAR-NBAIR-Ma16. These compounds have been extracted from *Melia dubia* leaves in earlier studies, where they served as effective feeding deterrents and insect repellents [36].

Our findings further highlight the complex and diverse nature of volatile organic compounds (VOCs) produced by *M. anisopliae* isolates. The type and quantity of these VOCs appear to be influenced by both the developmental stage of the fungus and the cultural conditions under which it is grown [11,22,33]. Such variability across entomopathogenic fungal isolates underscores their ecological significance and potential biocontrol value. Specific VOCs may attract insects, enhancing spore dispersal through a "lure and kill" mechanism, while others could act as repellents, potentially reducing the efficacy of the fungus as a biocontrol agent [25]. For instance, virulent strains of *M. anisopliae* have been shown to be avoided by pests such as sweet potato weevils and termites, due to the release of specific volatiles including 2-Nonanone, 2-Nonanol, and 3-Nonen-2-one [32]. In our study, these compounds were found in ICAR-NBAIR-Ma35, UASR BC-Ma26, and UASR BC-Ma60, further substantiating their role in fungal-insect interactions. A comparative analysis of percentage mortality and VOC profiles among the various isolates revealed notable differences in virulence. UASR BC-Ma38 exhibited the highest insecticidal efficacy, with a mortality rate of 98.89 % against *S. litura*, coupled with the presence of 28 distinct VOCs, many with proven insecticidal properties. In contrast, ICAR-NBAIR-Ma14 displayed considerably lower virulence, with a mortality rate of just 45.56 % and a comparatively smaller VOC profile consisting of only 14 compounds. This clear correlation between the chemical diversity of fungal metabolites and insect mortality highlights the importance of metabolite profiling in selecting effective fungal strains for biocontrol.

Overall, the study reaffirms the significant role of entomopathogenic fungal metabolites in pest management strategies. The wide array of bioactivities including insecticidal, ovicidal, feeding-deterrent, repellent, antioxidant, and antimicrobial effects makes them promising candidates for sustainable and environmentally friendly pest control. Importantly, the adoption of fungal metabolites as alternatives to synthetic insecticides may contribute to reduced production costs and decreased environmental impact. To fully capitalize on the potential of these fungal metabolites, however, further studies are essential. Future research should aim at isolating and characterizing the most effective insecticidal compounds, evaluating their toxicity profiles and conducting field trials to determine their practical efficacy under real-world conditions. This will ensure the development of safe, cost-effective, and ecologically viable pest control solutions based on secondary metabolites of *M. anisopliae*.

Table 1: Laboratory bioassay of native isolates of Metarhizium anisopliae against Spodoptera litura

Concentrations (Spores/mL)	No. of larvae	Per cent Mortality											
		INMa4	**INMa14**	**INMa16**	**INMa35**	**Ma2**	**Ma7**	**Ma26**	**Ma27**	**Ma31**	**Ma35**	**Ma38**	**Ma60**
$1x10^2$	30	37.78 (37.92)^{e}	18.89 (25.74)^{f}	30.00 (33.19)^{h}	40.00 (39.22)^{f}	42.22 (40.52)^{g}	30.00 (33.19)^{e}	22.22 (28.11)^{f}	26.67 (31.09)^{g}	25.56 (30.36)^{f}	38.89 (38.55)^{f}	33.33 (35.25)^{f}	48.89 (44.36)^{g}
$1x10^3$	30	40.00 (39.22)^{e}	21.11 (27.34)^{ef}	37.78 (37.90)^{g}	42.22 (40.52)^{f}	45.56 (42.45)^{ef}	33.33 (35.25)^{de}	26.67 (31.06)^{ef}	31.11 (33.90)^{f}	27.78 (31.77)^{ef}	43.33 (41.16)^{f}	37.78 (37.90)^{f}	54.44 (47.55)^{fg}
$1x10^4$	30	45.56 (42.45)^{e}	24.44 (29.62)^{de}	46.67 (43.09)^{f}	50.00 (45.00)^{e}	47.78 (43.73)^{ef}	36.67 (37.25)^{de}	30.00 (33.19)^{de}	36.67 (37.25)^{e}	32.22 (34.58)^{de}	53.33 (46.91)^{e}	48.89 (44.36)^{e}	57.78 (49.49)^{ef}
$1x10^5$	30	54.44 (47.56)^{d}	28.89 (32.50)^{cd}	53.33 (46.91)^{e}	56.67 (48.84)^{de}	51.11 (45.64)^{de}	41.11 (39.86)^{cd}	34.44 (35.90)^{d}	41.11 (39.88)^{d}	37.78 (37.92)^{d}	62.22 (52.08)^{d}	58.89 (50.12)^{e}	63.33 (52.75)^{de}
$1x10^6$	30	63.33 (52.73)^{c}	31.11 (33.87)^{c}	64.44 (53.40)^{d}	62.22 (52.08)^{cd}	55.56 (48.19)^{cd}	45.56 (42.44)^{bc}	42.22 (40.52)^{c}	45.56 (42.45)^{c}	44.44 (41.81)^{c}	73.33 (58.94)^{c}	72.22 (58.20)^{d}	67.78 (55.42)^{cd}
$1x10^7$	30	73.33 (58.94)^{b}	37.78 (37.92)^{b}	72.22 (58.20)^{c}	67.78 (55.42)^{c}	60.00 (50.78)^{bc}	53.33 (46.91)^{b}	46.67 (43.09)^{bc}	47.78 (43.73)^{c}	51.11 (45.64)^{c}	81.11 (64.26)^{b}	82.22 (65.08)^{c}	72.22 (58.20)^{bc}
$1x10^8$	30	80.00 (63.49)^{b}	41.11 (39.87)^{ab}	77.78 (61.89)^{b}	76.67 (61.15)^{b}	63.33 (52.75)^{ab}	63.33 (52.75)^{a}	53.33 (46.91)^{ab}	52.22 (46.27)^{b}	58.89 (50.12)^{b}	92.22 (73.88)^{a}	94.44 (76.52)^{b}	77.78 (61.89)^{b}
$1x10^9$	30	90.00 (71.73)^{a}	45.56 (42.45)^{a}	85.56 (67.69)^{a}	83.33 (65.97)^{a}	68.89 (56.10)^{a}	67.78 (55.44)^{a}	56.67 (48.84)^{a}	57.78 (49.48)^{a}	66.67 (54.75)^{a}	93.33 (75.04)^{a}	98.89 (86.49)^{a}	86.67 (68.68)^{a}
Control	30	0.00 (0.00)^{f}	0.00 (0.00)^{g}	0.00 (0.00)^{i}	0.00 (0.00)^{g}	0.00 (0.00)^{h}	0.00 (0.00)^{f}	0.00 (0.00)^{g}	0.00 (0.00)^{h}	0.00 (0.00)^{g}	0.00 (0.00)^{g}	0.00 (0.00)^{g}	0.00 (0.00)^{h}
SEm(±)		**1.040**	**0.670**	**1.013**	**1.017**	**0.839**	**0.855**	**0.767**	**0.803**	**0.903**	**1.136**	**1.164**	**1.081**
CD (1 %)		**4.234**	**2.727**	**4.125**	**4.138**	**3.415**	**3.480**	**3.124**	**3.269**	**3.676**	**4.623**	**4.736**	**4.400**
CV (%)		**3.916**	**3.878**	**3.927**	**3.882**	**3.440**	**3.884**	**3.889**	**3.864**	**4.306**	**3.927**	**3.996**	**3.844**

#INMa4=ICAR-NBAIR-Ma-4, INMa14=ICAR-NBAIR-Ma14, INMa16=ICAR-NBAIR-Ma16, INMa35=ICAR-NBAIR-Ma35, Ma2=UASR BC-Ma2, Ma7=UASR BC-Ma7, Ma26=UASR BC-Ma26, Ma27=UASR BC-Ma27, Ma31=UASR BC-Ma31, Ma35=UASR BC-Ma35, Ma38=UASR BC-Ma38 and Ma60=UASR BC-Ma60.

Figures in the parenthesis are arcsine values for per cent mortality

CD @ P= 0.01 significant at (0.01) per cent level of significance

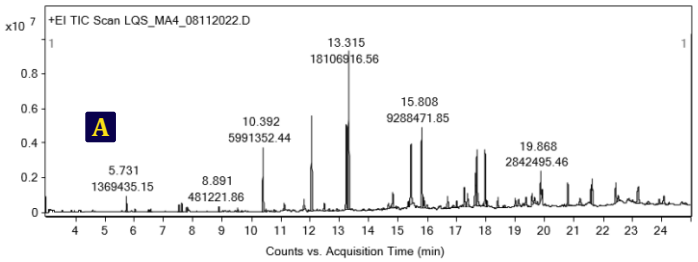
Per cent mortality followed by same letters in a column not significantly different by DMRT

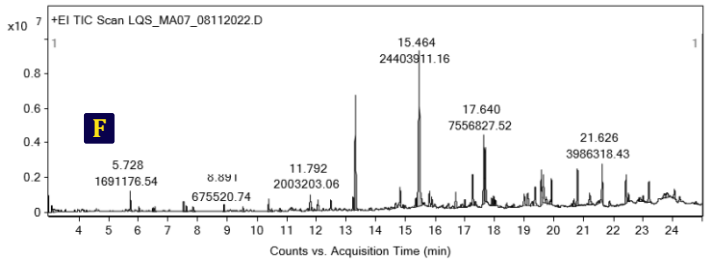
Table 2: Bioactive volatile organic compounds identified in crude extracts of 12 M. anisopliae isolates through GC-MS/MS analysis

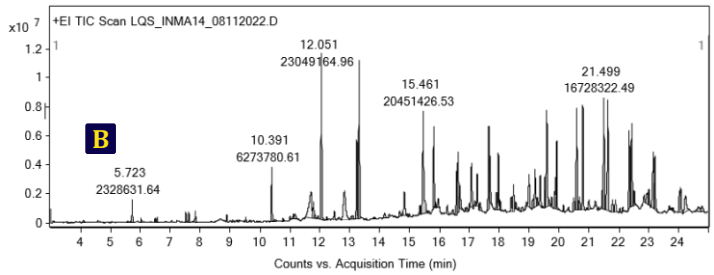
Sl. No.	Compound name	INMa4	INMa14	INMa16	INMa35	Ma2	Ma7	Ma26	Ma27	Ma31	Ma35	Ma38	Ma60
1	Ethanone, 1-(3-pyridinyl)	P	P	P	P	P	P	A	P	P	P	P	P
2	5-Aziridinopentanol	P	A	A	A	A	A	A	A	A	A	A	P
3	9,12-Octadecadienoic Acid (Z,Z)-	P	A	A	P	P	P	A	A	A	P	P	A
4	Toluene, 4-(1,1-dimethyl-2-propynyloxy)-	P	A	A	A	A	A	A	A	A	A	A	A
5	Acetamide, N-butyl-N-propyl-	P	A	P	P	P	P	P	P	P	A	P	P
6	Beryllium, bis(2,4-pentanedionato-O,O')-, (T-4)-	P	P	P	A	P	P	A	P	A	A	A	P
7	Tris(aziridinomethyl)hydrazine	P	P	P	P	P	A	P	P	P	A	P	P
8	Diethyl pyridine-3,4-dicarboxylate	P	P	P	P	P	P	P	P	P	P	P	P
9	Valeramide, N-butyl-N-propyl-	P	A	A	A	A	A	A	P	A	A	A	P
10	S-Allyl-L-cysteine, N-(n-butyl)-, n-butyl ester	P	P	P	P	A	P	P	P	P	P	P	P
11	1-(1-Azacyclopropyl)decane	P	A	A	A	A	P	A	A	A	A	P	A
12	N,N,N',N'-Tetraethyl-1,2-di-(thiophen-2-yl)ethane-1,2-diamin	P	A	A	A	A	A	A	A	A	A	A	A
13	Propanamide, N,N-diisopropyl-3-trimethylsilyl-	P	A	A	P	A	A	A	A	A	A	P	A
14	Methanesulphonamide, N-(2-pentyl)-N-ethyl-	P	P	P	P	P	P	P	P	P	P	P	P
15	Silane, diethyl(cis-4-methylcyclohexyloxy)pentyloxy-	P	A	A	A	P	A	A	A	A	P	A	A
16	2-[3-[N-Aziridyl]propyl]aminomethyltetrahydropyran	P	A	A	A	A	A	A	A	A	A	A	A
17	9,10-Anthracenedione, 1-amino-	P	A	P	P	P	P	A	A	P	P	A	P
18	Acetamide, N-butyl-N-pentyl-	P	A	A	A	P	A	A	A	A	A	P	A
19	1-(Diethylamino)-pentafluoropropene (Z)	P	A	A	A	A	A	A	P	A	A	P	P
20	N-[3-[N-Aziridyl]propyl]-3-dimethylaminopropylamine	P	P	P	P	P	P	P	P	P	P	P	P
21	Benzonitrile, 4-ethenyl-	A	A	P	P	P	P	A	P	A	P	P	P
22	Decyl heptyl ether	A	A	P	A	A	A	A	A	P	A	A	A
23	2-Aminobenzoyl hydrazide	A	A	P	A	A	A	A	A	P	P	A	A
24	Propionamide, 3-chloro-N-(2-butyl)-N-(3-methylbutyl)-	A	A	P	A	A	A	A	A	P	A	A	A
25	N-[3-[6-Hydroxyhexyl]aminopropyl]aziridine	A	A	P	A	A	P	A	P	P	A	A	A
26	Acetamide, 2-chloro-N-propyl-N-tetradecyl-	A	P	P	P	A	P	A	P	A	P	P	P
27	1,3-Propanediamine, N,N-diethyl-N'-methyl-	A	A	P	P	P	A	A	A	A	P	P	P
28	Carbonic acid, monoamide, N-propyl-N-(hept-2-yl)-, butyl est	A	A	P	A	A	A	A	A	A	A	A	A
29	Egtazic acid	A	A	P	A	A	P	A	A	A	A	A	A
30	Terbutaline, 3TMS derivative	A	A	P	A	A	A	A	P	A	A	A	A
31	N-[3-[4-Diethylamino-1-methylbutylamino]propyl]aziridine	A	A	P	A	A	A	A	A	A	A	A	A
32	Acetamide, N-butyl-N-hexyl-	A	A	P	A	A	A	A	A	P	A	A	P
33	1,2-Benzenedicarboxylic Acid, Diisooctyl Ester	A	A	P	A	A	A	A	A	A	A	A	A
34	Lauramide, N-butyl-N-(3-methylbutyl)-	A	A	P	A	A	A	A	P	A	A	A	A
35	Butyloctadecylamine	A	A	P	P	A	P	A	A	A	A	A	A
36	1,3-Propanediamine, N,N-diethyl-	A	P	A	A	P	A	P	A	A	A	A	A
37	N-[.beta.-Diethylaminoethyl]-N'-[7-chloro-4-quinolyl]diazene	A	P	A	A	A	A	A	A	A	A	A	A
38	Spermine	A	P	A	A	A	A	A	A	P	A	A	P
39	Anthraquinone, 2-amino-	A	P	A	A	A	A	A	P	A	A	A	A
40	Acetamide, N-methyl-N-hexadecyl-	A	P	A	P	P	P	A	A	A	P	A	P
41	4,5-Methylenedioxy-2-bromo-N-[1-methyl-4-diethylaminobu	A	P	A	A	A	A	A	A	A	A	A	A
42	Pyridine, 4-(1,1-dimethylethyl)-	A	A	A	P	P	A	A	A	A	P	P	A
43	Nonane, 4,5-dimethyl-	A	A	A	P	P	A	A	A	A	P	P	A
44	benzaldehyde, 4-(diethylamino)-2-methoxy-	A	A	A	P	A	A	P	A	A	A	P	A
45	1-(Hexahydropyrrolizin-3-ylidene)-3,3-dimethyl-butan-2-one	A	A	A	P	A	A	P	A	A	A	P	P
46	Valeramide, 5-chloro-N-(2-butyl)-N-butyl-	A	A	A	P	A	A	P	P	A	A	A	P
47	Bromacetamide, N-hexadecyl-	A	A	A	P	A	A	A	A	A	A	A	A
48	N'-[1-[4-Chlorophenyl]-1H-tetrazol-5-yl]-N,N-diethyl-1,3-pro	A	A	A	P	A	A	A	A	A	P	A	A
49	Propionamide, N-propyl-N-tetradecyl-	A	A	A	P	A	A	A	A	A	A	A	P
50	Propionamide, 3-bromo-N-propyl-N-dodecyl-	A	A	A	P	A	A	A	A	A	P	A	A
51	Benzenamine, N-ethyl-2-methyl-	A	A	A	A	P	A	A	A	A	A	P	A
52	Quinoline, 6-methyl-	A	A	A	A	P	A	A	A	A	P	A	A
53	N-[4-Aminobutyl]aziridine	A	A	A	A	P	A	A	A	A	A	A	A
54	N-[3-Methylaminopropyl]aziridine	A	A	A	A	P	P	A	A	A	A	A	A
55	4-Isobutyl-2-Pyrrolidione 1-TMS Deriv	A	A	A	A	P	A	A	A	A	A	A	A
56	1-(3-Aminopropyl)-2-pipecoline	A	A	A	A	P	A	A	P	A	A	A	A
57	1,3-Propanediamine, N,N'-bis(3-aminopropyl)-	A	A	A	A	P	A	A	A	A	A	A	A
58	Hexanamide, 2-bromo-N-(2-pentyl)-N-propyl-	A	A	A	A	P	A	A	A	A	P	A	A
59	Acetamide, N-butyl-N-3-methylbutyl-	A	A	A	A	A	P	A	A	A	P	A	A
60	Valeramide, 5-chloro-N-(2-butyl)-N-ethyl-	A	A	A	A	A	P	A	A	A	A	A	A
61	Ala-Gly, N-(n-propyloxycarbonyl)-, n-propyl ester	A	A	A	A	A	P	A	A	A	A	A	A
62	3,11-Diazatricyclo[7.3.1.0(3,8)]trideca-5,7-dien-4-one, 11-(2-hydroxyethyl)-	A	A	A	A	A	P	A	A	P	A	A	A

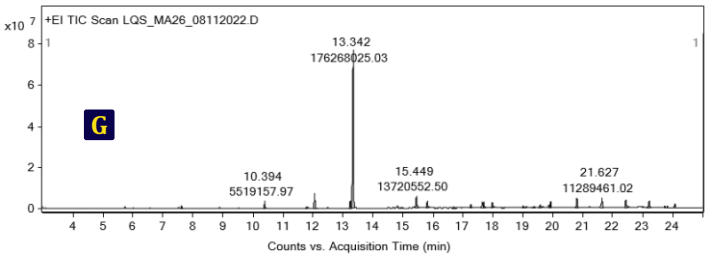
63	.beta.-[3-[Diethylaminopropyl]amino]propionitrile	A	A	A	A	A	P	A	A	A	A	A	A
64	N-[4-Cyclooctylaminobutyl]aziridine	A	A	A	A	A	A	P	A	A	A	A	A
65	Ethyl 3-(N-butylacetamido)propanoate	A	A	A	A	A	A	P	A	A	A	A	A
66	o-Tolidine, N,N'-bis(trimethylsilyl)-	A	A	A	A	A	A	A	P	A	A	P	A
67	Quinoline	A	A	A	A	A	A	A	A	P	A	A	A
68	3,4-Difluorobenzoic acid, 2- ethylhexyl ester	A	A	A	A	A	A	A	A	P	A	A	A
69	Propionamide, 3-chloro-N-(2- butyl)-N-(3-methylbutyl)-	A	A	A	A	A	A	A	A	P	A	A	A
70	Deoxyspergualin	A	A	A	A	A	A	A	A	P	A	A	A
71	1-Octen-3-ol	A	A	A	A	A	A	A	A	P	A	P	P
72	N,N',N''- Triethyldiethylenetriamine	A	A	A	A	A	A	A	A	P	A	P	A
73	3-Piperidinecarboxamide, N,N-diethyl-	A	A	A	A	A	A	A	A	P	A	A	A
74	1,4,8,11-Tetraazacyclotetradecane, 1,4,8,11-tetramethyl-	A	A	A	A	A	A	A	A	P	P	A	A
75	Cumidine	A	A	A	A	A	A	A	A	A	P	A	A
76	3,6-Diazahomoadamantan-9-ol	A	A	A	A	A	A	A	A	A	A	P	A
77	9-Eicosyne	A	A	A	A	A	A	A	A	A	A	P	A
78	2,3-Pentanedione 2-mono((3-ethyl-4-methyl-5-isoxazolyl)hyd	A	A	A	A	A	A	A	A	A	A	P	A

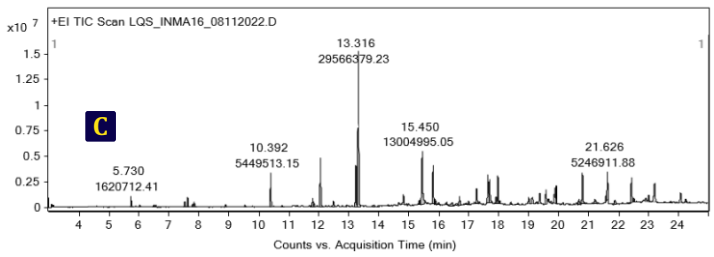
#INMa4=ICAR-NBAIR-Ma-4, INMa14=ICAR-NBAIR-Ma14, INMa16=ICAR-NBAIR-Ma16, INMa35=ICAR-NBAIR-Ma35, Ma2=UASR BC-Ma2, Ma7=UASR BC-Ma7, Ma26=UASR BC-Ma26, Ma27=UASR BC-Ma27, Ma31=UASR BC-Ma31, Ma35=UASR BC-Ma35, Ma38=UASR BC-Ma38 and Ma60=UASR BC-Ma60.
##A= Absent and P= Present

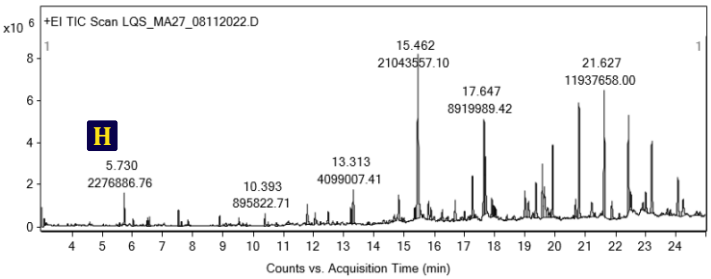


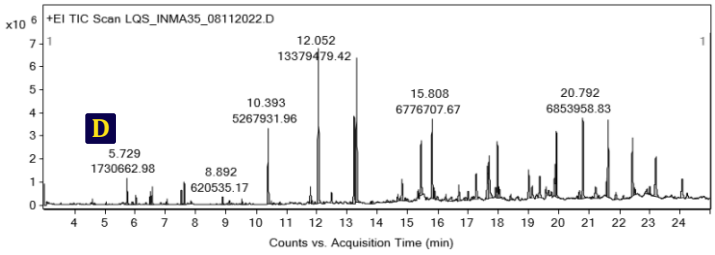


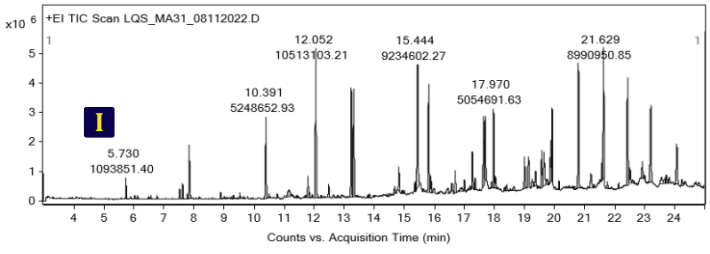


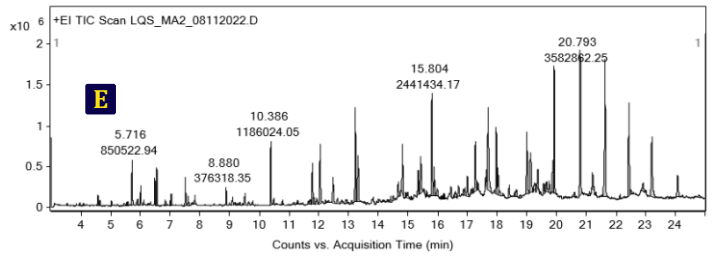


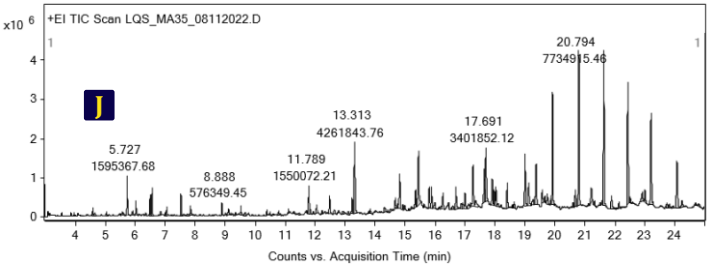












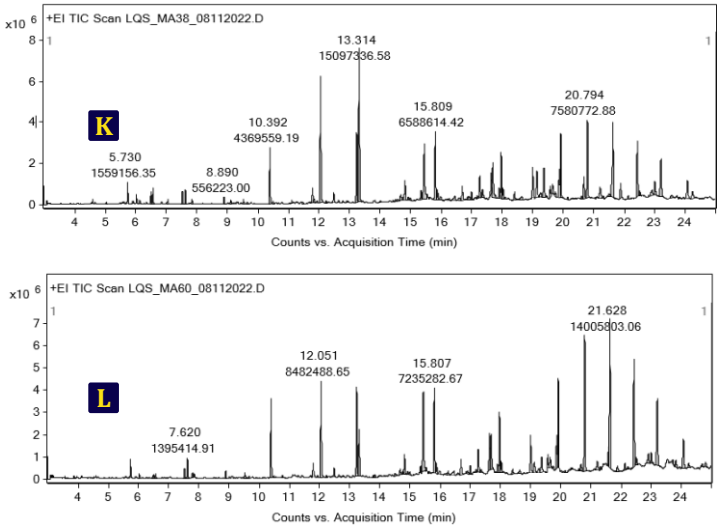


Fig. 1. GC-MS/MS Analysis of Crude Extract Mass Spectrum profiles from 12 Isolates of M. anisopliae; *(a) ICAR-NBAIR-Ma-4, (b) ICAR-NBAIR-Ma14, (c) ICAR-NBAIR-Ma16, (d) ICAR-NBAIR-Ma35, (e) UASR BC-Ma2, (f) UASR BC-Ma7, (g) UASR BC-Ma26, (h) UASR BC-Ma27, (i) UASR BC-Ma31, (j) UASR BC-Ma35, (k) UASR BC-Ma38 and (l) UASR BC-Ma60.*

Conclusion

In conclusion, the present study explored the efficacy of 12 isolates of *M. anisopliae* against *S. litura* and identified the volatile organic compounds (VOCs) produced by these isolates. The bioassay results demonstrated varying mortality rates, with isolate UASR BC-Ma38 exhibiting the highest efficacy at 98.89 %, surpassing the standard check ICAR-NBAIR-Ma4 and other isolates. Notably, the mortality rates increased with higher spore concentrations, emphasizing the concentration-dependent nature of the entomopathogenic effect. Gas Chromatography-Tandem Mass Spectrometry (GC-MS/MS) analysis of ethyl acetate crude extracts uncovered a diverse spectrum comprising 78 compounds across the isolates. Certain compounds, such as Diethyl pyridine-3,4-dicarboxylate, Methanesulphonamide, N-(2-pentyl)-N-ethyl-, and N-[3-[N-Aziridyl]propyl]-3-dimethylaminopropylamine, exhibited consistent presence in all isolates. Additionally, several compounds, including 1-Octen-3-ol, 1,2-Benzenedicarboxylic Acid, Diisooctyl Ester, 9-Eicosyne, Ethanone, 1-(3-pyridinyl), 5-Aziridinopentanol, Acetamide, N-butyl-N-propyl-, 9,10-Anthracenedione, 1-amino-, quinoline, 3,11-Diazatricyclo [7.3.1.0(3,8)] trideca-5,7-dien-4-one, 11-(2-hydroxyethyl)- and nonane derivatives, displayed insecticidal properties. The study highlights the importance of expanding the range of potential bioactive compounds with insecticidal and antimicrobial activities. These findings underscore the complex interplay of volatile compounds and their potential role in insect control. The study contributes valuable insights into the varied effects of *M. anisopliae* isolates on *S. litura* and highlights the diversity of bioactive compounds that could be harnessed for eco-friendly pest management. Future research endeavors should delve deeper into the field application of these fungal metabolites, considering factors such as production costs and ecological impact to establish their practical utility in integrated pest management strategies. Overall, this study lays the foundation for further exploration and utilization of *M. anisopliae* metabolites as effective and environmentally friendly alternatives for insect pest control.

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