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Research Article

Metabolome heterogeneity in the isolates of entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin

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ABSTRACT: Entomopathogenic fungi are known to produce a multitude of low molecular weight secondary metabolites involved in different biological processes including fungal development, intercellular communication and interaction with other organisms in complex niches. In the present investigation, heterogeneity in metabolome profile of three isolates of *Beauveria bassiana viz.,* MH590235 (TM), MK918495 (BR) and KX263275 (BbI8) were analyzed through GC-MS. Distinct differences in metabolite profile of the isolates were observed. A total of 63 metabolites were detected from all the isolates combined. Metabolites, 5-Oxotetrahydrofuran-2-carboxylic acid and undecane were found to be specific to BR isolate. Macrocyclic gamma lactones were detected in culture filtrates of BR and BbI8, oleic acid and hexadecanoic acid in TM and BR. An insecticidal compound, levoglucos an was detected in all the fungal isolates. Among the isolates, TM revealed higher variability in the metabolite production through PCA analysis. The metabolome of TM isolate contained compounds having several biological functions, *viz.,* insecticidal and antimicrobial activity, lipid and fatty acid metabolisms and virulence enhancing factors.

KEYWORDS: *Beauveria bassiana*, biological functions, GC-MS, metabolome heterogeneity, PCA Analysis

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INTRODUCTION

Entomopathogens are considered as a promising component of Integrated Pest Management Programmes (Butt, 2001) among which fungal Biocontrol Agents (BCAs) are widely exploited in view of their broad spectrum activity and amenability for mass production. All BCAs are known for the production of enzymes and secondary metabolites responsible for pathogenicity. The cuticle degrading enzymes, *viz.,* lipases, proteases and chitinases were targets of study from the time of discovery of mode of action of these fungal BCAs but descriptive studies on the secondary metabolite production by these agents are meagre.

Most often, the fungal BCAs secrete metabolites in extremely small quantities even under optimal conditions (Vey *et al*., 2001). Destruxins produced by *Metarhizium* spp. (Wahlman and Davidson, 1993), beauvericin and bassianolide by *Beauveria bassiana* (Xu *et al.,* 2008; Xu *et al.,* 2009), hirsutellin by *Hirsutella thompsonii* (Mazet and Vey, 1995) are the few metabolites widely studied. Little is known about the complete range of metabolites produced by most of the EPF. Though these fungi produce a wide array of bioactive compounds, the knowledge on specific role of a particular compound is lacking. Production of these metabolites may vary between genus, species and growth conditions (Kershaw *et al*., 1999; Amiri-Besheli *et al*., 2000; Wang *et al*., 2004).

Many studies have been conducted on virulence of several strains of *Beauveria* spp. on insect hosts, in particular, *B. bassiana* (Talaei-Hassanloui *et al*., 2006; Valero-Jiménez *et al.,* 2014). Few studies demonstrated variation in host range of fungus within species and between species of *Beauveria* (Rohrlich *et al.,* 2018). However, limited studies were carried out on the variation in metabolite profile among isolates of a particular species of fungal BCAs and hence the present study was undertaken to characterize variation in metabolite production among three isolates of *B. bassiana* grown under similar conditions.

MATERIALS AND METHODS

Cultures and growth conditions

Beauveria bassiana isolates bearing NCBI accessions MH590235, MK918495 and KX263275 were obtained from Department of Agricultural Entomology and Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. Pure cultures of the isolates were maintained at 28±5°C on Potato Dextrose Agar (PDA) medium for carrying out the study. Mycelial discs were cut from heavily sporulated culture plates using cork borer and inoculated into Potato Dextrose Broth (PDB) for extraction of metabolites.

Extraction of secondary metabolites

Isolates of *B. bassiana* were cultured in PDB for seven days after which culture filtrates were collected and adjusted to pH 2.0 with 37% (wt/vol) HCl. Metabolites were thrice extracted with an equal volume of ethyl acetate and the pooled ethyl acetate extracts of three biological replicates were dried using a rotary evaporator and re-suspended in HPLC grade methanol (1 ml). The extracts were then dried over Na_2SO_4 and evaporated under vacuum at 60º C to concentrate the metabolites. The metabolites were finally dissolved in HPLC grade methanol and utilized for GC-MS analysis (Strasser *et al.*, 2000).

Gas Chromatography- Mass Spectrometry (GC-MS)

The samples were analyzed using a model Clarus SQ 8C (Perkin Elmer) equipped with a MSD detector (Perkin Elmer). The GC injector port temperature was set to 220^{o}C, interface temperature at 250^{o}C and source temperature was set at 220^{o}C. The MS range was set to scan from 50 to 550 Da. The oven temperature was programmed to 75^{o}C (hold 2 min), then to 150^{o}C (10^o C/min), then to 250^{o}C (10^{o}C/min). The injection volume of 1.0 µl and split ratio of 1:12 and the injector used was split less mode. Helium was used as the carrier gas in constant-flow mode of 1.0 ml/min. The DB-5 MS capillary standard non - polar column (Agilent Co., USA) with dimensions were 0.25mm OD x 0.25µm ID x 30 m length was used for analysis. The MS source was maintained at 220^{o}C, 4.5e^{-6} motor vacuum pressure and ionization energy was set to -70eV. The MS have inbuilt pre-filter which reduced the neutral particles. Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST14). The spectrum of the unknown component was compared with the spectrum of the known components stored in the inbuilt library.

Identification of the metabolites were performed using spectra of individual components transferred to the NIST mass spectral search programs MS Search 2.2v where they were matched against the NIST MS library. Biological function of these compounds was identified by mapping all the metabolites in the KEGG database and Metaboanalyst 2.0.

Statistical analysis

Principal Component Analysis (PCA) and heatmap construction combined with hierarchical clustering were performed using JMP software (version 14) using the data from GC-MS. Percentage area values were used as independent variables in this multivariate analysis. Metabolites were clustered using R software for heat map generation.

RESULTS AND DISCUSSION

Culture filtrates of three isolates of *B. bassiana* were extracted using ethyl acetate and the variability in metabolite profile of different isolates of *Beauveria bassiana* were assessed using GC-MS (Fig. 1, 2, 3). In the present investigation, intraspecific variation was observed in the metabolites extracted from culture filtrates of the three isolates of *B. bassiana*. 29 metabolites including alkanes, carboxylic acid derivatives, glucopyranose and galactofuranose derivatives, unsaturated fatty acids, hexadecanoic acid derivatives were identified in TM isolate (Table 1, Fig. 1). 29 and 26 metabolites were detected in BR and BbI8 isolates mass spectrum respectively (Table 2, 3). Hyun *et al.* (2013) reported the presence of alcohols, amino acids, organic acids, phosphoric acids, purine nucleosides and bases, sugars, saturated fatty acids, unsaturated fatty acids, or fatty amides in 70 % methanol and 100 % hexane extracts of fruiting bodies of *Cordyceps bassiana*.

PCA is a powerful tool to selectively identify the major controlling factors contributing to differences between samples. It is hence applied in the present study for the comparative visualization and interpretations of the changes in the metabolites profiles of three *B. bassiana* isolates (Ramadan *et al.,* 2006).

PCA biplot for ethyl acetate extracts of three isolates of *B. bassiana* are presented in Figure 4. In the biplot, PCA 1 explained 52 % of the variation and PCA 2 explained 33.2 % of the variation. Results showed clear distinction of TM from other isolates. TM was separated alone in PC 1 while BR and BbI8 were separated from TM along PC 2. Higher levels of palmitic acid and oleic acid were obtained in TM compared to BR.

The present investigation showed distinct differences in metabolite profile of *B. bassiana* isolates (Fig. 5, 6). BR and BbI8 isolates showed similarities in the level of metabolite production (Fig. 4). An anhydrase, 1,6-anhydro-α-D-Glucopyranose (levoglucosan) was detected in all the three isolates. A gamma lactone, 5-Oxotetrahydrofuran-2-carboxylic acid was found to be present in the isolates, TM and BbI8. Syed *et al.* (2018) reported the insecticidal activity of levoglucosan obtained through pyrolysis of bio-oils against cutworm larvae.

The furan metabolite, 5-Oxotetrahydrofuran-2-carboxylic acid is a derivative of bassialone, an antimicrobial secondary metabolite produced by *B. bassiana* was detected in TM isolate in the present study. However, this was absent in BR isolate which showed clear variation in metabolite profile and this may indicate reduced virulence. 2-Deoxy-2-fluoro-1,6-anhydro-á-d-glucopyranose, 3-Hydroxy-2,3-dihydromaltol, 5-Hydroxymethylfurfural, Trioxsalen, Sucrose, Octadecanoic acid and 9,12-Octadecadienoic acid (Z,Z)- were detected in all the three isolates (Table 4) but the level of production varied among the isolates in terms of per cent area. This was confirmed through correlation analysis where positive significant correlation was detected between BR and BbI8 isolates of *B. bassiana* (Table 5, Fig. 7).

The metabolome of isolate TM was completely different from the other two isolates thus revealing least similarity with the other isolates (Fig. 5). Many studies were conducted in relation to the heterogeneity of secretome of entomopathogenic fungi under different growth conditions as well as extraction methods (Smedsgaard, 1997; Hyun *et al.,* 2013; Oh *et al.,* 2014). de Bekker *et al.* (2013) studied variation in metabolite production of *Metarhizium* and *Beauveria* during infectious and saprophytic growth.

Toxicity of secondary metabolites of *B. brongniartii* against pine caterpillar, *Dendrolimus tabulaeformis* was reported by Fan *et al*. (2008). Secondary metabolites of *B. brongniartii* was found to disable the immune mechanisms of *D. tabulaeformis*, and kill its host (Fan *et al*., 2013). In the present study, the metabolome of isolate TM was completely different from the other two isolates thus revealing least similarity with the other isolates (Fig. 5). In a previous study, isolate TM registered lowest values of LC50 (2.4 x 107 conidia ml-1) and LT50 (3.62 days) compared to the BR

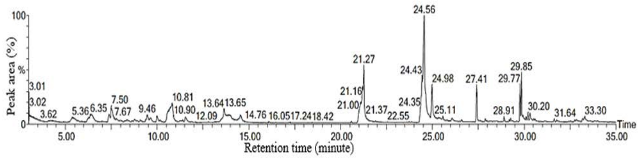

Fig. 1. **GC-MS chromatogram of secondary metabolites from *Beauveria bassiana* TM.**

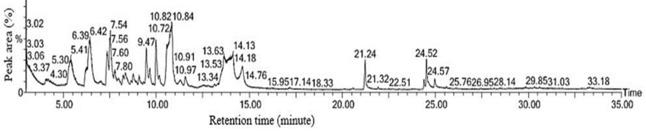

Fig. 2. **GC-MS chromatogram of secondary metabolites from *Beauveria bassiana* BR.**

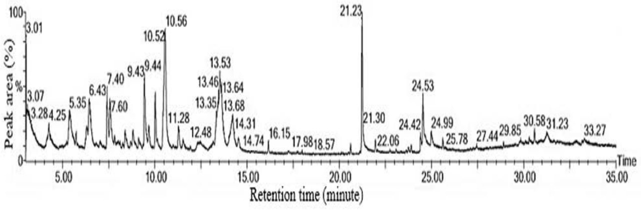

Fig. 3. **GC-MS chromatogram of secondary metabolites from *Beauveria bassiana* Bb18.**

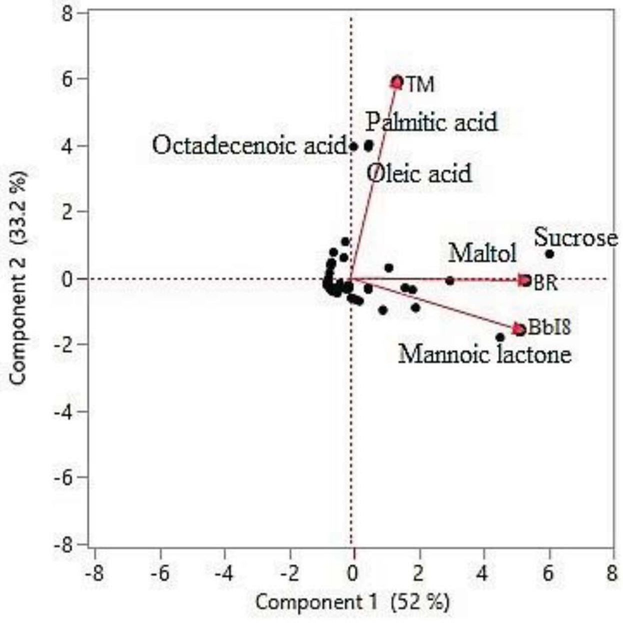

Fig. 4. **PCA biplot of three isolates of *Beauveria bassiana*.**

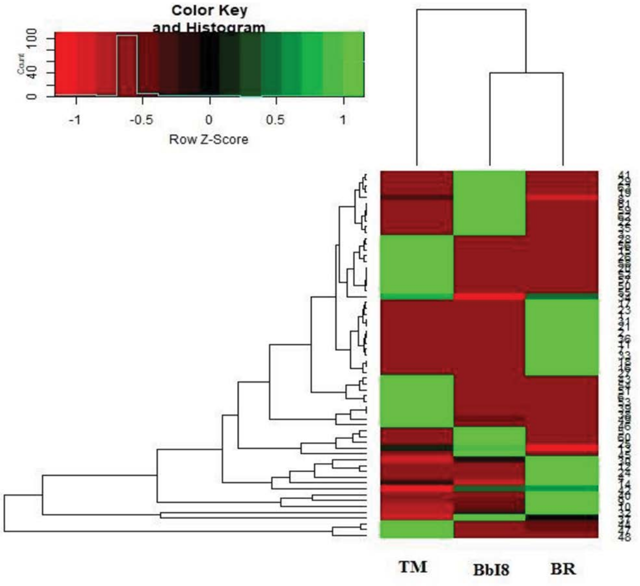

Fig. 5. **Heatmap and hierarchial clustering of GC-MS profiles of three isolates of *Beauveria bassiana*.**

Table 1. GC-MS based metabolite profile of *Beauveria bassiana* TM

Sl. No	Compound	RT	Area (%)	Molecular weight (g/mol)	Molecular formula	Biological action	Reference
1	Cyclohexanamine, N-3-butenyl-N-methyl-	5.449	1.857	221.388	$C_{15}H_{27}N$	Insecticidal, repellent, antimicrobial	Ibrahim *et al.,* 2001
2	Undecane	5.674	0.348	156.31	$C_{11}H_{24}$	Mild sex attractant of moths, alert signal for insects	Hölldobler and Wilson, 1990
3	2-Deoxy-2-fluoro-1,6-anhydro-á-d-glucopyranose	6.275	0.761	182.15	$C_6H_{11}FO_5$	Cell wall synthesis	Douglas, 2001
4	3-Hydroxy-2,3-dihydromaltol	6.395	2.744	128.13	$C_6H_8O_3$	-	-
5	5-Oxotetrahydrofuran-2-carboxylic acid	7.395	1.255	130.099	$C_5H_6O_4$	Bassianolone derivative	Oller-Lopez *et al.,* 2005
6	5-Hydroxymethylfurfural	7.500	3.083	126.11	$C_6H_6O_3$	Fermentation inhibitor	Kadowaki *et al.,* 2018
7	1,3-Oxathiolane, 2-methyl-2-iso-propyl-	7.795	0.686	146.250	$C_7H_{14}OS$	-	-
8	Cyclohexanone, 2-(2-butynyl)-	8.766	0.364	150.221	$C_{10}H_{14}O$	Antibacterial activity	Liu *et al.,* 2009
9	Sulfurous acid, cyclohexylmethyl undecyl ester	9.461	1.088	332.543	$C_{18}H_{36}O_3S$	Insecticidal	Domon *et al.,* 2018
10	1,3-Propanediol, 2-methyl-2-pro-pyl-	9.646	0.827	132.203	$C_7H_{16}O_2$	Lipid metabolosim	Liu *et al.,* 2015
11	Trioxsalen	9.991	0.836	228.24	$C_{14}H_{12}O_3$	Antimicrobial	Gowri *et al.,* 2011
12	Sucrose	10.832	8.207	342.297	$C_{12}H_{22}O_{11}$	Source for growth and spore production	Samsinakova, 1966
13	á-D-Glucopyranose, 1,6-anhydro-	11.542	0.838	162.141	$C_6H_{10}O_5$	Insecticidal	Syed *et al*., 2018
14	1,6-Anhydro-à-d-galactofuranose	13.663	4.054	162.141	$C_6H_{10}O_5$	Cell wall component	Bernabe *et al.,* 2011
15	2-Imidazolidinethione	13.908	4.060	102.158	$C_3H_6N_2S$	-	-
16	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	14.548	2.726	342.297	$C_{12}H_{22}$	Cell wall component	Bernabe *et al.,* 2011
17	Palmitic acid	21.271	16.273	256.43	$C_{16}H_{32}O_2$	Pesticidal activity, Lipid peroxidation	Vivekanadan *et al*., 2018
18	9,12-Octadecadienoic acid (Z,Z)-	24.447, 26.078	5.522	280.4	$C_{18}H_{32}O_2$	Fatty acid metabolism	Zhang *et al*., 2012
19	9-Octadecenoic acid, (E)-	24.562	15.968	282.4614	$C_{18}H_{34}O_2$	Fatty acid metabolism	Brennan *et al.,* 1975
20	Octadecanoic acid	24.977	3.766	284.48	$C_{18}H_{36}O_2$	Fatty acid metabolism	Zhang *et al.,* 2012
21	Ethyl linoleate	25.332	0.379	308.4986	$C_{20}H_{36}O_2$	Fatty acid metabolism	Zhang *et al.,* 2012
22	Glycidyl palmitate	27.413	2.950	312.494	$C_{19}H_{36}O_3$	Fatty acid metabolism	Zhang *et al.,* 2012
23	Eicosanoic acid, ethyl ester	28.909	0.363	340.592	$C_{22}H_{44}O_2$	Antimicrobial activity	Suresh *et al.,* 2014
24	Butyl linoleate	29.774	1.760	336.56	$C_{22}H_{40}O_2$	Fatty acid metabolism	Zhang *et al.,* 2012
25	Glycidyl oleate	29.854	2.540	338.532	$C_{21}H_{38}O_3$	Fatty acid metabolism	Zhang *et al*., 2012
26	1,3-Distearoylglycerol	30.204	0.436	568.924	$C_{35}H_{68}O_5$	Enhancement of virulence	Ortiz-Urquiza *et al*., 2016

27	2-Palmitoylglycerol	30.319	0.800	330.5026	$C_{19}H_{38}O_4$	Insecticidal	Nagalakshmi and Murthy, 2015
28	Digitoxin	32.855	0.370	764.95	$C_{41}H_{64}O_{13}$	Na-K ATPase inhibitor	PubChem 441201
29	Oleic anhydride	33.295	0.986	546.921	$C_{36}H_{66}O_3$	Fatty acid metabolism	Zhang *et al.*, 2012

Table 2. GC-MS based metabolite profile of *Beauveria bassiana* BR

Sl. No	Compound	RT	Area (%)	Molecular weight (g/mol)	Molecular formula	Biological action	Reference
1	2-Deoxy-2-fluoro-1,6-anhydro-á-d-glucopyranose	3.013	8.110	182.15	$C_6H_{11}FO_5$	Cell wall synthesis	Douglas, 2001
2	Dihydrothiophenone	3.574	0.729	102.151	C_4H_6OS	Insecticidal , nematicidal	Champagne *et al.*, 1986; Hudson and Toers, 1991
3	2-t-Butyl-5-propyl-[1,3] dioxolan-4-one	4.174	0.458	186.251	$C_{10}H_{18}O_3$	Fungitoxic	Horsefall and Lukens, 1965
4	Thymine	5.414	5.303	126.11	$C_5H_6N_2O_2$	Pyridine metabolism	Liu *et al.*, 2015
5	Nonane, 2-methyl-5-propyl-	5.664	0.559	184.367	$C_{13}H_{28}$	Insect growth regulator	Mian and Mulla, 1982
6	3-Hydroxy-2,3-dihydromaltol	6.425	9.919	128.13	$C_6H_8O_3$	-	-
7	Cyclohexane, 1,1'-dodecylidenebis [4-methyl-	7.040	0.431	362.6752	$C_{26}H_{50}$	Insecticidal, repellent, antimicrobial	Ibrahim *et al.*, 2001
8	(S)-(-)-1-Amino-2-(methoxymethyl)-pyrrolidine	7.365	2.820	130.19	$C_6H_{14}N_2O$	Antimicrobial	Dumoulin *et al.*, 2010
9	5-Hydroxymethylfurfural	7.500	3.083	126.11	$C_6H_6O_3$	Fermentation inhibitor	Kadowaki *et al.*, 2018
10	Coumarin-6-carboxaldehyde	7.770	1.326	174.155	$C_{10}H_6O_3$	Antimicrobial	Al-Majedy *et al.*, 2017
11	1-Decanamine	7.980	0.673	269.517	$C_{18}H_{39}N$	-	-
12	1-(Methylthio)-3-pentanone	8.331	1.330	132.23	$C_6H_{12}OS$	-	-
13	N-Nitroso-2,4,4-trimethyloxazolidine	8.766	0.831	144.172	$C_6H_{12}N_2O_2$	Antimicrobial, Anti-inflammatory	Kim *et al.*, 2001
14	2-Hydroxy-3-methylsuccinic acid	9.086	0.632	148.114	$C_5H_8O_5$	TCA cycle derivative	Hyun *et al.*, 2013
15	2,2-Dimethylcyclopropancarboxylic acid	9.466	2.422	114.14	$C_6H_{10}O_2$	-	-
16	Hydroxy-docosahexaenoic acid	9.666	1.114	344.5	$C_{22}H_{32}O_3$	Antibacterial	Mil-Homens *et al.*, 2012
17	Trioxsalen	9.986	2.949	228.24	$C_{14}H_{12}O_3$	Antimicrobial	Gowri *et al.*, 2011
18	1,2-Heptanediol	10.161	0.851	132.2	$C_7H_{16}O_2$	-	-
19	Sucrose	10.821	14.363	342.297	$C_{12}H_{22}O_{11}$	Source for growth and spore production	Samsinakova, 1966

20	Sumatriptan	11.297	0.519	295.402	$C_{14}H_{21}N_3O_2S$	Serotonin receptor agonist	NCBI, 2018
21	á-D-Glucopyranose, 1,6-anhydro-	11.552	0.787	162.1406	$C_6H_{10}O_5$	Insecticidal	Syed *et al*., 2018
22	Ethylenediamine-N,N'-dipropionic acid	13.317	0.456	204.226	$C_8H_{16}N_2O_4$	Insecticidal	Paulraj *et al.,* 2011
23	3-Deoxy-d-mannoic lactone	13.643	6.063	162.14	$C_6H_{10}O_5$	Fructose and mannose metabolism	Hyun *et al*., 2013
24	4,5-Dihydroxy-6-hydroxymethyl -oxepan-3-one	14.133	10.885	176.17	$C_7H_{12}O_5$	-	-
25	3-Deoxy-d-mannonic acid	14.643	5.113	180.156	$C_6H_{12}O_6$	Fructose and mannose metabolism	Hyun *et al*., 2013
26	Palmitic acid	21.236	1.907	256.43	$C_{16}H_{32}O_2$	Pesticidal activity, Lipid peroxidation	Vivekanadan *et al*., 2018
27	9,12-Octadecadienoic acid (Z,Z)-	24.402	0.447	280.4	$C_{18}H_{32}O_2$	Fatty acid metabolism	Zhang *et al*., 2012
28	Oleic Acid	24.522	1.866	282.47	$C_{18}H_{34}O_2$	Fatty acid metabolism	
29	Octadecanoic acid	24.972	0.504	284.48	$C_{18}H_{36}O_2$	Fatty acid metabolism	

Table 3. GC-MS based metabolite profile of *Beauveria bassiana* Bb18

Sl. No	**Compound**	**RT**	**Area (%)**	**Molecular weight (g/mol)**	**Molecular formula**	Biological action	Reference
1	Undecane	4.249	1.086	184.37	$C_{13}H_{28}$	Mild sex attractant of moths, alert signal for insects	Hölldobler and Wilson, 1990
2	2-Nonadecanone 2,4-dinitrophenylhydrazine	4.334	0.552	462.635	$C_{25}H_{42}N_4O_4$	-	-
3	Clindamycin	5.389	2.804	424.98	$C_{18}H_{33}ClN_2O_5S$	Antibiotic	Woappi *et al.,* 2016
4	2-Deoxy-2-fluoro-1,6-anhydro-á-d-glucopyranose	6.315	1.322	182.15	$C_6H_{11}FO_5$	Cell wall synthesis	Douglas, 2001
5	3-Hydroxy-2,3-dihydromaltol	6.435	4.010	128.13	$C_6H_8O_3$	-	-
6	5-Oxotetrahydrofuran-2-carboxylic acid	7.400	1.985	130.099	$C_5H_6O_4$	Bassianolone derivative	Oller-Lopez *et al*., 2005
7	5-Hydroxymethylfurfural	7.555	1.544	126.11	$C_6H_6O_3$	Fermentation inhibitor	Kadowaki *et al*., 2018
8	1,2,3-Butanetriol	8.391	0.951	106.121	$C_4H_{10}O_3$	-	-
9	2-Methoxy-4-vinylphenol	8.821	0.776	150.177	$C_9H_{10}O_2$	-	-
10	3-Propylglutaric acid	9.441	3.237	174.196	$C_8H_{14}O_4$	-	-
11	1,3-Dioxane-5-methanol, 4,5-dimethyl-	9.676	1.094	146.186	$C_7H_{14}O_3$		
12	Trioxsalen	10.021	1.679	228.24	$C_{14}H_{12}O_3$	Antimicrobial	Gowri *et al*., 2011
13	Sucrose	10.556	9.462	342.297	$C_{12}H_{22}O_{11}$	Source for growth and spore production	Samsinakova, 1966
14	á-D-Glucopyranose, 1,6-anhydro-	11.532	0.568	162.141	$C_6H_{10}O_5$	Insecticidal	Syed *et al.,* 2018
15	Benzocycloheptano[2,3,4-I ,j]isoquinoline, 4,5,6,6 atetrahydro-1,9-dihydroxy-2,10-dimethoxy-5-methyl-	12.482	0.584	341.407	$C_{20}H_{23}NO_4$	-	

16	3-Deoxy-d-mannoic lactone	13.528	12.451	162.14	$C_6H_{10}O_5$	Fructose and mannose metabolism	Hyun *et al.*, 2013
17	3-Deoxy-d-mannonic acid	14.228	4.823	180.156	$C_6H_{12}O_6$	Fructose and mannose metabolism	Hyun *et al.*, 2013
18	d-Glycero-d-galacto-heptose	14.533	1.143	210.182	$C_7H_{14}O_7$	-	-
19	Propanamide, 2-(3,5-dioxopiperazin-1-yl)-3-phenyl-	21.241	5.571	393.912	$C_{20}H_{28}ClN_3O_3$	-	-
20	9,12-Octadecadienoic acid (Z,Z)-	24.417	0.573	280.4	$C_{18}H_{32}O_2$	Fatty acid metabolism	Zhang *et al.*, 2012
21	cis-Vaccenic acid	24.532	2.442	282.468	$C_{18}H_{34}O_2$	-	-
22	Octadecanoic acid	24.987	0.668	284.48	$C_{18}H_{36}O_2$	Fatty acid metabolism	Zhang *et al.*, 2012
23	Methly palmitate	30.314	0.807	444.647	$C_{28}H_{44}$	Fatty acid metabolism	Zhang *et al.*, 2012
24	Diisooctyl phthalate	30.584	0.807	390.564	$C_{24}H_{38}O_4$	Insecticidal	(Wakil *et al.*, 2017
25	1,2-Dipalmitin	30.940	0.818	568.924	$C_{35}H_{68}O_5$	Insecticidal	Ragavendran *et al.,* 2017
26	Ethyl iso-allocholate	31.235	1.008	436.633	$C_{26}H_{44}O_5$		

Table. 4. Comparison of metabolites of different isolates of *Beauveria bassiana*

Sl. NO.	**Compound**	**Isolatesof** Beauveria bassiana		
		TM	BR	B10
1	5-Oxotetrahydrofuran-2-carboxylic acid	+	-	+
2	á-D-Glucopyranose, 1,6-anhydro-	+	+	+
3	Undecane	+	-	+
4	2-Deoxy-2-fluoro-1,6-anhydro-á-d-glucopyranose	+	+	+
5	3-Hydroxy-2,3-dihydromaltol	+	+	+
6	5-Hydroxymethylfurfural	+	+	+
7	Trioxsalen	+	+	+
8	Sucrose	+	+	+
9	3-Deoxy-d-mannoic lactone	-	+	+
10	3-Deoxy-d-mannonic acid	-	+	+
11	n-Hexadecanoic acid	+	+	-
13	Octadecanoic acid	+	+	+
14	9,12-Octadecadienoic acid (Z,Z)-	+	+	+
15	Oleic Acid	+	+	-
+	Detected			
-	Not detected			

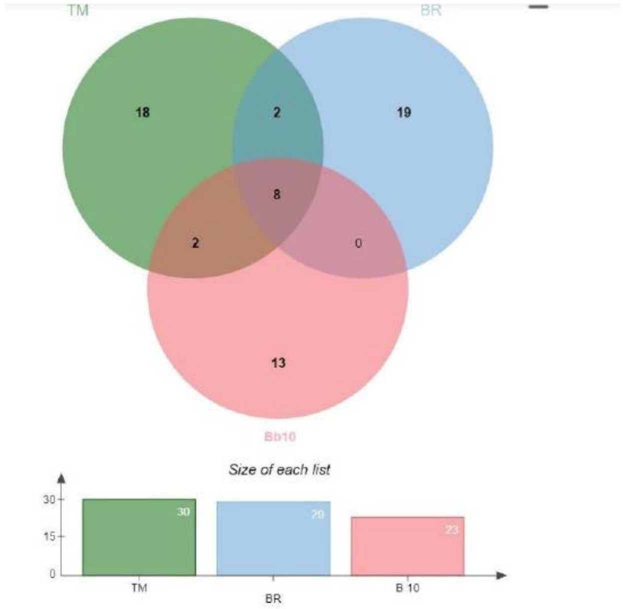

Fig. 6. Venn diagram representing the GC-MS profile of *Beauveria bassiana* isolates.

(Nithya *et al*., 2019). The enhanced virulence of TM may be attributed to the distinctive metabolites involved in lipid and fatty acid metabolisms. These metabolites might have enabled the fungus to overcome the action of detoxifying enzymes inside insects such as esterases and glutathione-S-transferases which take part in defense responses against the fungus.

In this study, non-targeted profiling approach was performed using GC-MS for metabolite profiling of three isolates of *B. bassiana.* The metabolite profile varied within the species and distinct profiles were recorded in the three study isolates, TM, BR and BbI8. So far, there are no reports on the correlation of metabolites between different isolates of *B. bassiana* and hence the results of the study can be used to interpret the pathogenicity of different isolates of entomopathogenic fungus against any host insect paving way for its management.

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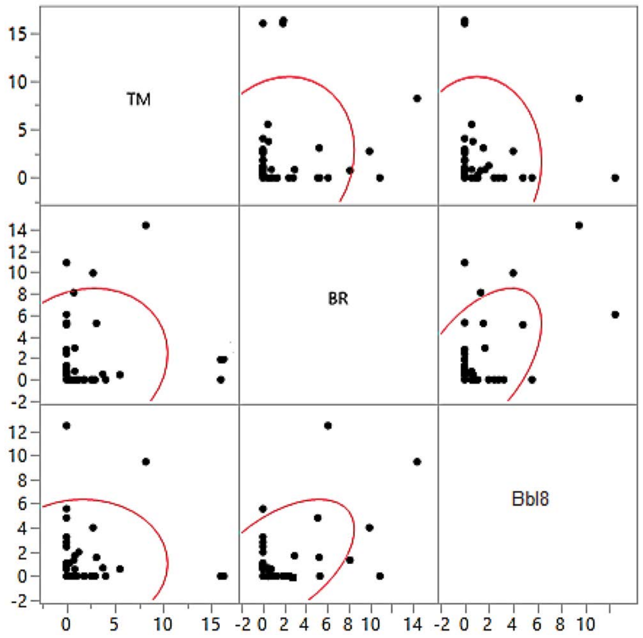

Fig. 7. Scatterplot matrix illustrating the pairwise correlation between the *Beauveria bassiana* isolates.

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