Isolation, purification and identification of biological compounds from *Beauveria* sp. and their evaluation as insecticidal effectiveness against *Bemisia tabaci*

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Abstract

Bemisia tabaci is one of the most notorious agricultural pests in the world. A vicious circle among insect resistance, dose increased, environment and human body impaired as the overuse of synthetic pesticides are becoming increasingly evident. Entomopathogenic *Beauveria* sp. is known as an effective natural enemy to control *B. tabaci*. Therefore, this study aimed to purify and identify the biological compounds from *Beauveria* sp. LY2 via extensive chromatographic techniques, NMR and MS and evaluated for their insecticidal activities against *B. tabaci* via contact and stomach toxicity assay. The outcome identified that one new cerebroside, cerebroside F (**1**), nine known compounds, cerebroside B (**2**), bassiatin (**3**), methyl 1,4-dihydro-4-oxo-2-quinolinecarboxylate (**4**), cerevisterol (**5**), 9-hydroxycerevisterol (**6**), 6-dehydrocerevisterol (**7**), (22E,24R)-ergosta-8(14),22-diene-3 β ,5 α ,6 β ,7 α -tetrol (**8**), melithasterol B (**9**) and ergosterol peroxide (**10**) were isolated. Among the known compounds, methyl 1,4-dihydro-4-oxo-2-quinolinecarboxylate (**4**) was isolated from natural origin for the first time. It is evident from the results that compounds **3**, **4** and **7** strongly featured insecticidal activities against *B. tabaci*, being the LC₅₀ value as 10.59, 19.05, 26.59 μ g/mL respectively in contact as well as 11.42, 5.66, 5.65 μ g/mL respectively in stomach experiment. The data from the current study has provided the foundation for the use of newly purified compounds against *Bemisia tabaci* as an alternative to synthetic chemical compounds.

Introduction

The whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae) is one of the world's top 100 invasive species which can attack more than 600 agricultural plant-host species under field and greenhouse conditions ^{1,2}. Due to its piercing-sucking mouthpart, *B. tabaci* can directly cause plant weakness and indirectly transmit approximately 111 plant viruses ³. Both nymphs and adults can also secrete honeydew, which can induce coal pollution. Besides B biotype (MEAM1) is the dominant species which significantly threatened many agricultural commodities in numerous countries ⁴⁻⁶. Moreover, for the excessive use of synthetic pesticides have contributed to resistance development of *B. tabaci*. So far, 64 active ingredients have been reported for resistance from *B. tabaci* ⁷.

The injudicious and excessive use of broad-spectrum synthetic pesticide can do harm to human health and environment ⁸, and it can also result in the development of heritable resistance, pest resurgence and secondary pest problems. By contrast, bio-insecticides are safer for humans and more environmentally friendly. Moreover, plant and fungi are the sources of thousands of secondary metabolites ⁹. Those secondary metabolites are ideal substitutes of those chemicals which play a significant role and possess a great potential on the field of biopesticides ¹⁰.

Beauveria sp. (Ascomycota: Hypocreales) is a facultative entomopathogen with an extremely broad spectrum which is used as a commercial biopesticide against many agriculturally important insect pests ¹¹. The metabolites from *Beauveria* sp. can be divided into three main kinds, such as alkaloids (tenellin, bassiatin, pyridovercin, pyridomacrolidin, ilicidin H), cyclodepsipetides (beauvericins, allobeauvericins,

bassianolides, beauveriolides) and benzoquinone (oosporein), many of them exert insecticidal ^{12,13}, anthelmintic ¹⁴, synergistic antifungal ¹⁵, antibacterial ^{16,17}, antiviral ¹⁸ and cytotoxic activities ¹⁹. However, research on the secondary metabolites of *Beauveria* sp. has a history of nearly 60 years, but the research on its activity is mainly focused on the field of medicinal use, and there are very few activities in the field of agriculture, in particular insecticidal activity. Although some studies have been conducted in isolation and identification of various compounds from natural resources, but, there is no research of bassiatin in the agricultural field per se. Therefore, this research study not only supplements the gap in the insecticidal activity of bassiatin, but also adds two types of substances to the group of secondary metabolites of *Beauveria* sp., and evaluates their insecticidal effects in both stomach and contact toxicity effects.

Results

2.1. Extraction, Separation and Purification of Extract

After silica gel column chromatography, gel chromatography and HPLC, compounds **1-10** were obtained. Among them, compound **1** is a new cerebroside compound, **4** is a new natural product, and **2-10** are all confirmed to be known compounds after comparison with previous studies, they are cerebroside B (**2**) ²⁰, bassiatin (**3**) ²¹, methyl 1,4-dihydro-4-oxo-2-quinolinecarboxylate (**4**) ^{22,23}, cerevisterol (**5**) ²⁴, 9-hydroxycerevisterol (**6**) ²⁵, 6-dehydrocerevisterol (**7**) ²⁶, (22*E*,24*R*)-ergosta-8(14),22-diene-3 β ,5 α ,6 β ,7 α -tetrol (**8**) ²⁷, melithasterol B (**9**) ²⁸, ergosterol peroxide (**10**) ²⁸. Compounds **1-2**, **4-9** are all isolated from *Beauveria* sp. for the first time. The Chemical structures of all the purified compounds (1-10) are presented in Figure 1.

2.2. Structure Elucidation of Compounds

Compound **1**, white amorphous powder, possessed a molecular formula of C₄₁H₇₅NO₁₀ by the positive HR-ESI-MS (*m/z* 742.5457 [M+H]⁺, calcd. 742.5469), requiring five indices of hydrogen deficiency. The ¹H NMR spectrum (Table 1) in methanol-*d*₄ of **1** showed two terminal olefinic protons at δ_H 5.79, 6.09 (each 1H, br s, H-19), two mutually coupled trans olefinic protons at δ_H 5.74 (1H, dtd, *J* = 15.4, 6.6, 0.7 Hz, H-5), 5.50 (1H, dtd, *J* = 15.4, 7.3, 1.3 Hz, H-4), the diagnostic anomeric proton of β -glucopyranoside moiety at δ_H 4.26 (1H, d, *J* = 7.8 Hz, H-1''), as well as two long-chain terminal methyl triplets at δ_H 0.89 (6H, t, *J* = 7.0 Hz, H-18, H-16'). The ¹³C NMR spectrum (Table 1) exhibited an α,β -unsaturated carbonyl carbon at δ_C 203.0 (s, C-8), an amide acyl carbon at δ_C 177.2 (s, C-1'), four olefinic carbon signals, a set of characteristic carbons corresponding to a β -D-glucopyranoside moiety appeared at δ_C 104.7 (d, C-1''), 75.0 (d, C-2''), 77.9 (d, C-3''), 71.6 (d, C-4''), 78.0 (d, C-5'') and 62.7 (t, C-6''), other three oxygenated carbons at δ_C 73.0 (d), 72.7 (d) and 69.7 (t), as well as a series of long-chain aliphatic carbon signals. The above NMR features were similar to those of cerebroside B (**2**) ²⁰, also isolated from the current study, and the obvious difference between them only came from the olefinic methyl part of the main chain, in which the olefinic methyl signal was disappeared and replaced by a terminal olefinic methylene in **1**, in addition, a

keto carbonyl at δ_C 203.0 was newly detected. In the HMBC spectrum (Figure S3), the correlations from H-19 [δ_H 5.79, 6.09 (each 1H, br s)] to the keto carbonyl carbon confirmed the presence of α , β -unsaturated carbonyl moiety. Furthermore, the correlations from H-7 [2.82 (2H, br t, J = 7.4 Hz)] to C-5 [133.4 (d)] and C-8 [203.0 (s)] revealed the keto carbonyl at C-8. In view of their consistency of the chemical shifts and coupling constants, the configurations of C-2, C-3 and C-2' were deduced to be the same as those of **2**. The interpretation of the MS fragment ions (see Figure S8 in supplementary material) allowed to determine the specific length of the two chains. Therefore, the structure of compound **1** was established as illustrated in Figure 2 and named as cerebroside F.

Table 1. The ^1H NMR and ^{13}C NMR spectral data of the compound **1** in methanol- d_4

No.	^1H NMR	^{13}C NMR	No.	^1H NMR	^{13}C NMR
1	3.70 (1H, dd, 10.4, 3.6) 4.10 (1H, dd, 10.4, 5.6)	69.7 (t)	19	5.79, 6.09 (each 1H, br s)	125.2 (t)
2	3.98 (1H, m)	54.6 (d)	1'	—	177.2 (s)
3	4.13 (1H, br dd, 7.5, 7.3)	72.7 (d)	2'	3.97 (1H, m)	73.0 (d)
4	5.50 (1H, ddt, 15.4, 7.3, 1.3)	131.7 (d)	3'	1.54, 1.71 (each 1H, m)	35.9 (t)
5	5.74 (1H, dtd, 15.4, 6.6, 0.7)	133.4 (d)	4'	1.35–1.45 (2H, m)	26.2 (t)
6	2.26–2.33 (2H, m)	28.1 (t)	5'–13'	1.24–1.33 (18H, m)	30.5–30.8 (t)
7	2.82 (2H, br t, 7.4)	38.1 (t)	14'	1.27 (2H, m)	33.1 (t)
8	—	203.0 (s)	15'	1.30 (2H, m)	23.7 (t)
9	—	150.2 (s)	16'	0.89 (3H, t, 7.0)	14.4 (q)
10	2.25 (2H, br t, 7.6)	32.0 (t)	1''	4.26 (1H, d, 7.8)	104.7 (d)
11	1.38 (2H, m)	29.8 (t)	2''	3.18 (1H, dd, 9.2, 7.8)	75.0 (d)
12–15	1.24–1.33 (8H, m)	30.5–30.8 (t)	3''	3.35 (1H, dd, 9.2, 8.8)	77.9 (d)
16	1.27 (2H, m)	33.1 (t)	4''	3.27 (1H, m)	71.6 (d)
17	1.30 (2H, m)	23.7 (t)	5''	3.27 (1H, m)	78.0 (d)
18	0.89 (3H, t, 7.0)	14.4 (q)	6''	3.66 (1H, dd, 11.9, 5.5) 3.86 (1H, dd, 11.9, 1.3)	62.7 (t)

Compound **2**, white amorphous powder, $C_{41}H_{77}NO_9$, HR-ESI-MS (m/z 728.5625 $[M+H]^+$). 1H NMR (s, 600 MHz, DMSO) δ 7.39 (d, J = 9.3 Hz, 1H, NH), 5.59 (m, 1H, H-5), 5.50 (d, J = 5.2 Hz, 1H, OH-2'), 5.40 (dd, J = 15.4, 6.8 Hz, 1H, H-4), 5.09 (d, J = 6.6 Hz, 1H, H-8), 4.96 (d, J = 4.1 Hz, 1H, OH-3), 4.93 (d, J = 5.5 Hz, 1H, OH-3''), 4.90 (dd, J = 7.9, 5.0 Hz, 2H, OH-2'', OH-4''), 4.51 (t, J = 5.9 Hz, 1H, OH-6''), 4.12 (d, J = 7.8 Hz, 1H, H-1''), 3.99 (dd, J = 12.9, 6.6 Hz, 1H, H-1), 3.92 (dd, J = 10.4, 5.8 Hz, 1H, H-3), 3.81 (m, 2H, H-2, H-2'), 3.66 (dd, J = 10.0, 6.1 Hz, 1H, H-6''), 3.52 (dd, J = 10.3, 3.7 Hz, 1H, H-1), 3.43 (dt, J = 11.7, 5.9 Hz, 1H, H-6''), 3.13 (td, J = 8.8, 4.8 Hz, 1H, H-3''), 3.08 (d, J = 5.8 Hz, 1H, H-5''), 3.04 (dd, J = 8.8, 5.2 Hz, 1H, H-4''), 2.95 (td, J = 8.4, 4.2 Hz, 1H, H-2''), 1.98 (m, 4H, H-6, H-7), 1.92 (t, J = 7.5 Hz, 2H, H-10), 1.55 (d, J = 14.5 Hz, 3H, Me-9), 1.47 – 1.14 (overlap, m, 40H, CH_2 -11~17, CH_2 -3'~15'), 0.86 (s, m, 6H, Me-18, Me-16'). ^{13}C NMR (151 MHz, DMSO) δ 174.14 (C-1'), 135.26 (C-9), 131.47 (C-5), 131.39 (C-4), 123.86 (C-8), 103.89 (C-1''), 77.28 (C-5''), 76.93 (C-3''), 73.78 (C-2''), 71.41 (C-2'), 70.93 (C-1), 70.39 (C-4''), 69.11 (C-3), 61.44 (C-6''), 53.22 (C-2), 39.43 (C-10), 34.86 (C-3'), 32.54 (C-6), 31.69 – 29.07 (overlap, C-12~16, C-5'~14'), 27.75 (C-7), 27.62 (C-11), 24.92 (C-4'), 22.49 (C-15'), 16.09 (C-19), 14.31 (overlap, C-18, C-16').

Compound **3**, colorless crystal, $C_{15}H_{19}NO_3$, ESI-MS (m/z 261.00 $[M]^+$) 1H NMR (600 MHz, $CDCl_3$) δ 7.33 (overlap, m, 3H, H-13, H-14, H-15), 7.13 (overlap, m, 2H, H-12, H-16), 4.41 (t, J = 4.4 Hz, 1H, H-3), 3.24 (dd, J = 37.0, 4.4 Hz, 1H, H-10), 3.03 (s, 3H, N-Me), 3.01 (d, J = 2.2 Hz, 1H, H-6), 2.30 (hd, J = 6.9, 2.2 Hz, 1H, H-7), 0.85 (d, J = 7.1 Hz, 3H, Me-8), 0.77 (d, J = 6.8 Hz, 3H, Me-9). ^{13}C NMR (151 MHz, $CDCl_3$) δ 167.38 (s, C-2), 165.64 (s, C-5), 134.26 (s, C-11), 129.89 (overlap, s, C-12, C-16), 129.34 (overlap, s, C-13, C-15), 128.33 (s, C-14), 81.38 (s, C-6), 62.87 (s, C-3), 37.19 (s, C-10), 32.53 (s, N-Me), 29.77 (s, C-7), 18.68 (s, C-8), 15.24 (s, C-9).

Compound **4** is white amorphous powder, $C_{11}H_9NO_3$, ESI-MS (m/z 202.20 $[M-H]^-$). 1H NMR (600 MHz, $CDCl_3$) δ 9.26 (s, 1H, H-N), 8.36 (d, J = 8.1 Hz, 1H, H-5), 7.67 (t, J = 7.7 Hz, 1H, H-7), 7.48 (d, J = 8.3 Hz, 1H, H-8), 7.39 (t, J = 7.6 Hz, 1H, H-6), 6.99 (s, 1H, H-3), 4.04 (s, 3H, H-10). ^{13}C NMR (151 MHz, $CDCl_3$) δ 179.55 (C-4), 163.40 (C-9), 138.96 (C-1a), 136.10 (C-2), 133.10 (C-7), 126.34 (C-4a), 126.27 (C-5), 124.51 (C-6), 117.97 (C-8), 111.65 (C-3), 53.76 (C-10).

Compound **5**, white amorphous powder, $C_{28}H_{46}O_3$, ESI-MS (m/z 431.20 $[M+H]^+$). 1H NMR (600 MHz, $CDCl_3$) δ 5.36 (m, 1H, H-7), 5.23 (dd, J = 15.3, 7.5 Hz, 1H, H-23), 5.16 (dd, J = 15.3, 8.2 Hz, 1H, H-22), 4.08 (td, J = 11.3, 5.7 Hz, 1H, H-3), 3.62 (d, J = 5.0 Hz, 1H, H-6), 1.09 (s, 3H, Me-19), 1.03 (d, J = 6.6 Hz, 3H, Me-21), 0.92 (d, J = 6.8 Hz, 3H, Me-28), 0.83 (dd, J = 9.3, 6.8 Hz, 6H Me-26, Me-27), 0.60 (s, 3H, Me-18). ^{13}C NMR (151 MHz, $CDCl_3$) δ 144.03 (C-8), 135.39 (C-22), 132.20 (C-23), 117.56 (C-7), 75.98 (C-5), 73.69 (C-6), 67.74 (C-3), 56.01 (C-17), 54.77 (C-14), 43.78 (C-13), 43.49 (C-9), 42.83 (C-24), 40.40 (C-20), 39.49 (C-4), 39.24 (C-12), 37.16 (C-20), 33.09 (C-25), 32.98 (C-1), 30.88 (C-2), 27.91 (C-16), 22.90 (C-15), 22.06 (C-11), 21.13 (C-21), 19.96 (C-26), 19.65 (C-27), 18.85 (C-19), 17.60 (C-28), 12.34 (C-18).

Compound **6**, white amorphous powder, $C_{28}H_{46}O_4$, HR-ESI-MS (m/z 915.6686 $[2M+Na]^+$). 1H NMR (600 MHz, MeOD) δ 5.36 (dd, J = 5.2, 2.3 Hz, 1H, H-7), 5.26 (m, 2H, H-22, H-23), 4.03 (m, 1H, H-3), 3.68 (dd, J = 5.2, 2.5 Hz, 1H, H-6), 2.53 (m, 1H, H-14), 1.15 (s, 3H, Me-19), 1.07 (t, J = 6.2 Hz, 3H, Me-21), 0.98 (t, J = 7.2

Hz, 3H, Me-28), 0.89 (dd, $J = 11.6, 6.8$ Hz, 6H, Me-26, Me-27), 0.69 (s, 3H, Me-18). ^{13}C NMR (151 MHz, MeOD) δ 144.65 (C-8), 137.84 (C-22), 134.17 (C-23), 121.89 (C-7), 79.77 (C-5), 76.95 (C-9), 74.57 (C-6), 69.03 (C-3), 58.24 (C-17), 52.74 (C-14), 45.68 (C-13), 45.22 (C-24), 42.70 (C-20), 42.28 (C-10), 41.61 (C-4), 37.39 (C-12), 35.24 (C-25), 32.50 (C-2), 30.06 (C-11), 30.03 (C-16), 29.12 (C-1), 24.87 (C-15), 23.09 (C-19), 22.50 (C-21), 21.33 (C-26), 20.95 (C-27), 19.07 (C-28), 13.13 (C-18).

Compound **7**, white amorphous powder, $\text{C}_{28}\text{H}_{44}\text{O}_3$, ESI-MS (m/z 429.20 $[\text{M}+\text{H}]^+$). ^1H NMR (600 MHz, CDCl_3) δ 5.65 (s, 1H, H-7), 5.24 (dd, $J = 15.3, 7.7$ Hz, 1H, H-23), 5.16 (dd, $J = 15.3, 8.4$ Hz, 1H, H-22), 4.03 (m, 1H, H-3), 2.52 (m, 1H, H-13), 1.04 (d, $J = 6.6$ Hz, 3H, Me-21), 0.96 (d, $J = 5.8$ Hz, 3H, Me-19), 0.92 (d, $J = 6.8$ Hz, 3H, Me-28), 0.83 (dd, $J = 9.6, 6.8$ Hz, 6H, Me-27, Me-26), 0.61 (s, 3H, Me-18). ^{13}C NMR (151 MHz, CDCl_3) δ 198.31 (C-6), 165.32 (C-8), 135.02 (C-22), 132.51 (C-23), 119.71 (C-7), 77.81 (C-5), 67.50 (C-3), 56.05 (C-17), 55.82 (C-14), 44.78 (C-9), 43.90 (C-13), 42.82 (C-24), 40.46 (C-20), 40.28 (C-12), 38.85 (C-4), 36.51 (C-10), 33.06 (C-25), 30.37 (C-2), 30.23 (C-1), 29.71 (C-16), 27.86 (C-16), 22.49 (C-11), 21.96 (C-15), 21.11 (C-21), 19.95 (C-26), 19.65 (C-27), 17.58 (C-19), 16.43 (C-28), 12.70 (C-18).

Compound **8**, white amorphous powder, $\text{C}_{28}\text{H}_{46}\text{O}_4$, ESI-MS (m/z 447.10 $[\text{M}+\text{H}]^+$). ^1H NMR (600 MHz, CDCl_3) δ 5.20 (qd, $J = 15.3, 7.3$ Hz, 2H, H-22, H-23), 4.42 (s, 1H, H-6), 3.91 (m, 1H, H-3), 3.14 (d, $J = 3.5$ Hz, 1H, H-7), 2.61 (dt, $J = 17.0, 8.4$ Hz, 1H, H-15), 1.02 (d, $J = 6.7$ Hz, 3H, Me-21), 0.91 (t, $J = 7.3$ Hz, 3H, Me-25), 0.87 (m, 6H, Me-18, Me-19), 0.83 (dd, $J = 9.9, 6.8$ Hz, 6H, Me-27, Me-28). ^{13}C NMR (s, 151 MHz, CDCl_3) δ 152.62 (C-14), 135.27 (C-22), 132.26 (C-23), 125.19 (C-8), 68.69 (C-3), 67.80 (C-5), 65.08 (C-6), 61.35 (C-7), 56.83 (C-17), 42.98 (C-24), 42.85 (C-13), 39.58 (C-20), 39.25 (C-3), 38.74 (C-9), 36.60 (C-12), 35.85 (C-10), 33.10 (C-26), 32.20 (C-1), 31.11 (C-2), 27.18 (C-16), 24.96 (C-15), 21.24 (C-21), 19.97 (C-28), 19.67 (C-27), 19.00 (C-11), 18.07 (C-19), 17.62 (C-18), 16.53 (C-25).

Compound **9**, white amorphous powder, $\text{C}_{28}\text{H}_{44}\text{O}_3$, ESI-MS (m/z 451.30 $[\text{M}+\text{Na}]^+$). ^1H NMR (600 MHz, CDCl_3) δ 5.19 (dq, $J = 15.3, 7.7$ Hz, 2H, H-22, H-23), 4.22 (s, 1H, H-7), 3.95 (m, 1H, H-3), 3.31 (d, $J = 2.6$ Hz, 1H, H-6), 1.14 (s, 1H, H-19), 1.02 (d, $J = 6.6$ Hz, 3H, Me-21), 0.91 (d, $J = 6.8$ Hz, 3H, Me-28), 0.83 (dd, $J = 9.5, 6.8$ Hz, 6H, Me-26, Me-27), 0.59 (s, 3H, Me-18). ^{13}C NMR (s, 151 MHz, CDCl_3) δ 135.56 (C-22), 134.47 (C-9), 132.01 (C-23), 126.94 (C-8), 68.57 (C-3), 67.12 (C-7), 65.66 (C-5), 62.62 (C-6), 49.60 (C-14), 42.84 (C-24), 42.09 (C-13), 40.41 (C-20), 39.16 (C-4), 38.00 (C-10), 35.68 (C-12), 33.10 (C-25), 30.84 (C-2), 30.20 (C-1), 29.01 (C-16), 23.85 (C-15), 23.41 (C-11), 22.81 (C-19), 20.96 (C-21), 19.97 (C-27), 19.65 (C-26), 17.66 (C-28), 11.29 (C-18).

Compound **10**, white amorphous powder, $\text{C}_{28}\text{H}_{44}\text{O}_3$, ESI-MS (m/z 429.20 $[\text{M}+\text{H}]^+$). ^1H NMR (600 MHz, CDCl_3) δ 6.50 (d, $J = 8.5$ Hz, 1H, H-7), 6.24 (d, $J = 8.5$ Hz, 1H, H-6), 5.22 (dd, $J = 15.2, 7.7$ Hz, 1H, H-23), 5.14 (dd, $J = 15.3, 8.4$ Hz, 1H, H-22), 3.97 (m, 1H, H-3), 1.00 (d, $J = 6.6$ Hz, 3H, Me-21), 0.91 (d, $J = 6.8$ Hz, 3H, Me-28), 0.88 (s, 3H, Me-19), 0.83 (d, $J = 6.8$ Hz, 3H, Me-27), 0.82 (overlap, m, 6H, Me-18, Me-26). ^{13}C NMR (151 MHz, CDCl_3) δ 135.44 (s, C-6), 135.22 (s, C-22), 132.32 (s, C-23), 130.76 (s, C-7), 82.17 (s, C-5), 79.44 (s, C-8), 66.46 (s, C-3), 56.20 (s, C-17), 51.69 (s, C-14), 51.09 (s, C-9), 44.57 (s, C-13), 42.78 (s, C-24),

39.74 (s, C-20), 39.35 (s, C-12), 36.97 (s, C-4), 36.93 (s, C-10), 34.70 (s, C-1), 33.07 (s, C-25), 30.11 (s, C-2), 28.65 (s, C-16), 23.40 (s, C-11), 20.88 (s, C-21), 20.63 (s, C-15), 19.95 (s, C-27), 19.65 (s, C-26), 18.18 (s, C-19), 17.56 (s, C-28), 12.87 (s, C-18).

2.3 In-vitro Insecticidal activity of isolated compounds

After the isolation, purification and identification, compounds **1-10** were evaluated for the insecticidal activity against *B. tabaci*. The results of the mean mortality via both contact and stomach toxicity was exhibited from Figure 3 to figure 6. Furthermore, the results of the LC₅₀ value at 72 h were presented in Table 2. However, the detail data was placed in supplementary material as table S1 to Table S10.

According to the mortality results of compound **1**, 41.67 % mortality was reported at 72 h and 50 ppm via contact assay. Similarly, 38.33% mortality was displayed at the same concentration and time exposure via stomach assay (Figure 3). Similar results for insecticidal activity was afforded by compound **2** by both bioassays i.e. 47.50 and 44.17 % mortality was recorded at 50 ppm via contact as well as stomach assay respectively (Figure 3). Results apparent from Figure 4 represents that compound **3** has higher mortality as 97.50 and 79.17 % at 50 ppm with exposure of 72 and 48 h by stomach assay. Similarly, by contact toxicity assay 79.17 and 54.17 % mortality was exerted at the same concentration and time. Moreover, significant mortality was also afforded at 25 ppm by stomach as well as contact assay i.e. 80.83; 66.67 % and 66.67; 46.67 % mortality at 72 and 48 h respectively. Similar results for mortality were also displayed by compound **4**, where, 100 and 85.00 % mortality was reported at 50 ppm at exposure of 72 and 48 h respectively. Similarly, 92.50, 76.66 and 66.67 % mortality was displayed at 25, 12.5 and 6.5 ppm at 72 h respectively. However, via contact assay maximum 87.50 % mortality was recorded at 72 h and 50 ppm concentration (Figure 4). Results for insecticidal activity of compound **5** are presented in (Figure 5) which showed that 70.83 and 66.67 % mortality was displayed at 72 h at the concentration of 50 and 25 ppm respectively by stomach toxicity assay. Similarly, by contact assay 53.33 and 50.83% mortality was recorded at the same concentration and time exposure respectively. Same trend of mortality was exerted by compound **6** where 41.67 and 36.67 % mortality was recorded at 50 ppm and time exposure of 72 h ((Figure 5)). Alternatively, compound **7** was the best compound that presented significantly high mortality i.e. 100 and 85.00 % mortality via stomach assay at 50 ppm concentration with 72 h exposure. Similarly, at 25 ppm 92.50 and 71.67 % mortality was recorded at the same exposure period. Interestingly, this compound displayed 66.67% mortality at 72 h exposure even at low concentration (6.5 ppm). Whereas, relatively lower mortality i.e. 71.67 and 65.83 % was recorded by contact assay at 50 and 25 ppm and 72 h exposure respectively (Figure 5). Results presented in (Figure 6) for compound **8** described that maximum mortality was afforded at 50 and 25 ppm with 72 and 48 h as 90.00 and 81.67 % respectively by stomach toxicity assay, whereas, at the same concentration and exposure period 61.67 and 57.56 % mortality was recorded by contact assay. Interestingly, compound **9** produced higher mortality on using contact assay rather than stomach assay like other described compounds. However, this compound produced moderate mortality in both bioassay methods i.e. 66.67; 51.67 and 64.17; 50.83 at 50 ppm at 72 and 48 h exposure respectively (Figure 6). On the other hand, compound **10** recorded relatively higher mortality as 80.83 and 68.33 % by contact toxicity assay at 50

and 25 ppm and 72 h exposure period respectively as compared to contact toxicity assay which afforded 56.67 and 46.83 % mortality at the same concentration and exposure period.

In essence, compound **3** exhibited an extraordinary mortality against *B. tabaci* on both contact and stomach experiments. Compound **4** and **7** highlighted the significant insecticidal activities on stomach bioassay and strong insecticidal activities on contact bioassay while compound **5, 8** and **10** demonstrated their strong stomach toxicity effectiveness while their contact insecticidal activities were moderate. Comparing to the values above, compounds **1, 2, 6** and **9** ranged from moderate to fair effectiveness to *B. tabaci*.

Table 2. Probit analysis of the effects of compounds **1-10** on *B. tabaci* at 72 h

	Compound	LC ₅₀	95 % F.L		Slope± SE	χ ²
			lower	upper		
contact	1	> 50	36.33	777.49	0.96±0.34	0.42
	2	> 50	40.42	265.48	0.99±0.21	0.05
	3	10.59	4.74	23.64	0.92±0.18	1.02
	4	19.05	13.74	26.40	0.88±0.07	5.60
	5	> 50	22.75	313.89	0.89±0.29	2.03
	6	> 50	22.83	3788.71	1.00±0.57	0.01
	7	26.59	16.80	42.07	0.96±0.10	0.83
	8	> 50	6.87	593.56	0.95±0.49	0.18
	9	41.52	20.34	84.76	0.99±0.16	0.10
	10	> 50	13.90	1548.93	0.92±0.53	0.42
stomach	1	> 50	6.82	52764.85	0.95±0.99	0.24
	2	> 50	36.52	228.20	0.98±0.20	0.72
	3	11.42	8.51	15.34	0.99±0.07	0.74
	4	5.66	3.38	9.47	0.99±0.11	0.37
	5	22.29	12.35	40.24	0.99±0.13	0.17
	6	> 50	23.66	1704.02	0.98±0.47	0.34
	7	5.65	3.38	9.47	0.99±0.11	0.36
	8	13.83	10.32	18.53	0.99±0.06	1.06
	9	> 50	27.75	149.46	0.81±0.19	3.30
	10	22.06	16.36	29.74	0.97±0.07	1.25

F.L (Fiducial Limit); X²; (Chi-square); SE (Standard Error); LC₅₀ (Lethal Concentration)

Probability analysis showing the LC₅₀ values, slope value, Chi-square and fiducial limits at 95% confidence limit. Lowest LC₅₀ values displayed by compounds **3**, **4** and **7** as 11.42, 5.66 and 5.65 µg/mL and 10.59, 19.05 and 26.59 µg/mL respectively via stomach and contact assay which illustrates their extraordinary insecticidal activity against *B. tabaci*. Whereas, other compounds displayed higher LC₅₀ values via stomach as well as contact assay respectively that showed their less toxicity (Table 2).

Discussion

Several problems are associated with the use of synthetic chemicals for pest management, the introduction of natural products for this purpose is the primary concern. Essential oils, extracts and biologically active compounds are commonly used due to their effectiveness and safety for the environment as well as human beings. Secondary metabolites or natural products isolated from botanical source and range of microorganisms displayed bioactivity such as insecticidal, microbial, fungicidal and cytotoxic properties and contribute to their survival in several ways³⁰. However, in the current research study all isolated compounds were evaluated for their in-vitro insecticidal activity against *Bemisia tabaci*. Different compounds displayed variable insecticidal activities from excellent to moderate activity.

The toxicity results of spinasterol, 22,23-dihydrospinasterol reported by Maqsood et al. are in accordance with the results that documented LC₅₀ values of 32.36 and 44.49 µg / mL by contact as well as residual assay respectively against *Brevicoryne brassicae*¹⁰. R.M. Zolotar et al. screened out ecdysteroids and were evaluated for their insecticidal activity against the Colorado beetle (*Leptinotarsa decemlineata*) using contact feeding method, that showed significant activity for isolated compounds³¹. The isolation of appropriate sterol from the *Beauveria* sp. and its activity against *B. tabaci* are in accordance with the findings of R.M. Zolotar et al. Similar results on insecticidal activity of ergosterol peroxide isolated from *Nomuraea rileyi* against tobacco cutworm were documented by Pannipa Prompiboon et al.³² who reported the moderate effects such as 46.7 % mortality was caused against *Spodoptera litura* larvae via topical application at an interval of one week with 90-120 µg / insect, whereas, our research results showed 38.33 % mortality at 72 h exposure at concentration of 50 ppm on *B. tabaci*. Additional biological activities of compounds ergosterol peroxide include antioxidant activities³³, antimycobacterial activities³⁴ inflammatory activities³⁵. Furthermore, the research study did not find any published material with the same compounds that was isolated on insecticidal activity study. That suggests there is still great potential of prospects on finding new active molecules from known entomopathogenic fungi.

B. tabaci is a potent agricultural pest with strong tendency to develop resistance against known pesticides, therefore new controlling agents are in constant need. In our study methyl 1,4-dihydro-4-oxo-2-quinolinecarboxylate (**4**), bassiatin (**3**), 6-dehydrocervisterol (**7**) have shown promising insecticidal activity against *B. tabaci*. Compounds **1**, **2**, **4-9** are discovered in *Beauveria* sp. for the first time and compound **4** was discovered in natural products for the first time. Compound **4** was previously synthesized by Mazzoni O.²⁹, even this is a known compound, we still use 2D NMR to fix the structure, due to no ¹³C NMR data was reported in literature.

Although different biological control approaches have been employed in current agriculture system to control pest on crops and vegetables, however, the use of fungal based biological compounds against *B. tabaci* is limited. The outcomes from this current study offered that isolated compounds from *Beauveria* sp. useful for the control of sucking pest especially *B. tabaci*. Hence, the introduction of these biologically active compounds could be an effective alternative and potential means to control such pests.

Materials And Methods

4.1. General Experimental Procedures

NMR spectra were captured on an Avance-600 NMR spectrometer (Bruker, 57 Karlsruhe, Germany) at room temperature. High-resolution electrospray ionization mass spectrometry (HRESIMS) spectra data were recorded on a 6500 series quadrupole-time-of-flight (Q-TOF) mass spectrometer (Agilent, Santa Clara, CA). Mass spectrometry also recorded on LCMS 8050 (Shimadzu, Tokyo, Japan). High-performance liquid chromatography (HPLC) analysis was performed on a 1260 Infinity LC system (Agilent, Santa Clara, CA), and the column used was a 250 mm × 4.6 mm i.d., 5 μm, ZORBAX Eclipse XDB (Agilent, Santa Clara, CA). Semipreparative HPLC was performed on a 1260 series system (Agilent), and the column used was a 250 mm × 9.4 mm i.d., 5 μm, ZORBAX Eclipse XDB (Agilent). Column chromatography was performed using silica gel (100–200 mesh) (Qingdao Ocean Chemical Co. Ltd., Qingdao, China) and Sephadex LH-20 (GE Healthcare, Uppsala, Sweden). All chemical reagents were purchased from a chemical reagent company (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) and used without further purification.

4.2. Fermentation and Extraction of *Beauveria* sp. LY2

The *Beauveria* sp. LY2 was obtained from the Laboratory of Microbial Metabolites, College of Plant Protection of Shenyang Agricultural University, China, which was cultured on SDAY medium at 25°C in an incubator. The fermentation has two stages where the first stage, SDY medium (1 % peptone, 1 % yeast extract, 4 % glucose, pH = 7.2) was used. Put a 0.5 cm diameter fungi pancake with SDAY medium into a 250 mL Erlenmeyer flask which contains 40 mL of SDY medium, then incubated them at 25°C with 180 rpm shaking speed for 3 days as to prepare the seed culture. In the second stage, Czapek–Dox medium (0.03 % NaNO₃, 0.01 % K₂HPO₄·3H₂O, 0.005 % MgSO₄·7H₂O, 0.005 % KCl, 0.0001 % FeSO₄·7H₂O, 3 % sucrose, pH = 7.0) was used. 120 2L Erlenmeyer flasks, each contains 400 mL Czapek–Dox medium, were inoculated with 40 mL seed culture at 25 °C with 180 rpm shaking speed for 15 days. The fermentation cultures were centrifuged at 4 °C with 5000 rpm for 30 mins to remove mycelia, then add 3 % Amberlite XAD 16 resin into the broth at 25°C with 180 rpm shaking speed for 4 hours. Resin was collected by using 100 meshes gauze and extracted four times with methanol. The dried crude extract was harvested through reduced pressure concentration. The dried methanol extract was dissolved in 600 mL solution (50 % CH₃OH, 50 % H₂O), the solution was extracted four times by 600 mL CH₂Cl₂. Collecting the resulted extract CH₂Cl₂ solution then concentrate it to get 10.0 g solid brown residue.

4.3. Isolation and Purification

The concentrated extract was purified by using silica gel chromatography to elute stepwise with CH₂Cl₂-MeOH (100:0, 50:1, 25:1, 10:1 and 0:100, 1.5 L each) as the mobile phase to afford six fractions, A to G. C was separated via silica gel chromatography with PE:EA (9:1, 4:1, 7:3, 6:4 and 1:1, 300 mL each) as the mobile phase to yield compound **3** (110 mg), compound **9** (7.3 mg) and compound **10** (14.4 mg). Fraction

E was subjected to gel chromatography on Sephadex LH-20 eluted with CH₂Cl₂-MeOH (1:1) to obtain compound **5** (16.7 mg). D, F, G was purified by reverse-phase semi-preparative HPLC applying a MeOH-H₂O gradient (contain 0.1 % HCOOH) respectively. Compound **6** (2 mg) was gained from D with 90% MeOH on 40 min (see Figure S26), compound **7** (4 mg) and compound **8** (4.2 mg) were also obtained from D with 85 % MeOH on 18 min and 20 min (see Figure S30 and Figure S34). Compound **4** (3.1 mg) was yielded from F with 77 % MeOH on 28 min (see Figure S19), **1** (3.8 mg) and **2** (25.9 mg) was obtained from G with conditions with 98 % MeOH on 10 min and 16 min respectively (see Figure S7 and Figure S12).

4.4. Evaluation of Insecticidal Activity against *B. tabaci*

4.4.1. *Bemisia tabaci* Culture

The whiteflies B-biotype *B. tabaci* (MEAM1) are from Liaoning Key Laboratory of Economic and Applied Entomology was cultured in a controlled greenhouse at 25-27 °C, 60 %-70 % relative humidity (RH) with 16 : 8 (light : dark) photoperiod, on around 2 months old cotton plants, *Gossypium hirsutum*.

4.4.2. Pre-experiment Container Preparation

One side of a lightproof bi-pass glass tube (3 cm inside diameter and 6 cm height) was covered with a stretched Parafilm M membrane (the first layer). Another side sealed with one piece of 100 meshes gauze and a rubber band³⁶. All items had to be completely sterile.

4.4.3. Experiment of Stomach Insecticidal Activity to *B. tabaci*

The whitefly diet comprised of 30 % (w / v) sucrose with serial dilutions of each compound (50, 25, 12.5, 6.25 µg / mL respectively), each diet contained 0.5 % Tween 80 and 0.002 % DMSO. Diet aliquots (200 µL) containing compounds were dispensed on top of the first layer of stretched Parafilm membrane over each container; another stretched Parafilm membrane was placed over the diet aliquots to prevent evaporation. 30 adults (2-5 d old) were aspirated from *G. hirsutum* into the container. Test containers were placed in a controlled greenhouse at 25 - 27 °C, 60 % -70 % under 16 : 8 (light : dark) photoperiod and mortality was recorded at 24, 48 and 72 h. Each iteration of the experiment consisted of four replicates for each concentration of each compound (4 replicates x 4 concentrations x 3 repetitions x 30 whiteflies per tube = 1440 *B. tabaci* per test compound).

4.4.4. Experiment of Contact Insecticidal Activity to *B. tabaci*

Used mixed solvent (methanol: dichloromethane = 1:1) to diluent each compound into serial concentrations (50, 25, 12.5, 6.25 µg/mL respectively), mixed solvent with compounds aliquots were dispensed into the container tube, rotated the tube once to let the solvent equally distributed and evaporate. 30 adults (2-5 d old) were aspirated from *G. hirsutum* into the container. Test containers were put in the controlled greenhouse at 25-27 °C, 60-70 % under 16: 8 (light : dark) photoperiod. *B. tabaci* mortality was recorded at 24, 48 and 72 h. Each iteration of the experiment consisted of four replicates

for each concentration of the each compound (4 replicates x 4 concentrations x 3 repetitions x 30 individual per tube = 1440 *B. tabaci* per test compound).

4.5. Statistical Analysis

All the calculated data on mortality was analyzed by one way analysis of variance (ANOVA), the mean difference between treatments was envisioned for significance test by Duncan multiple range test DMRT with IBM-SPSS statistics 25.0 version software. Probit analysis was performed using EPA Probit analysis program version 1.5. 0.

4.6. Figure software

All the chemical structures including bold bonds and arrows were drawn with ChemDraw 19.0. The Figures 3-6 were drawn with Origin 2019b (9.65).

Conclusions

The present research study indicated that the secondary metabolites from *Beauveria* sp. possess potential botanical agents. Results also demonstrated that *B. tabaci* produced significant sensitivity to isolated bassiatin (**3**) via both stomach and contact bioassay as well as methyl 1,4-dihydro-4-oxo-2-quinolinecarboxylate (**4**) and 6-dehydrocervisterol (**7**) via stomach bioassay. cervisterol (**5**), (22*E*,24*R*)-ergosta-8(14),22-diene-3 β ,5 α ,6 β ,7 α -tetrol (**8**), melithasterol B (**9**) and ergosterol peroxide (**10**) displayed moderate toxicity against this pest. In contrast, cerebrosides F (**1**) and cerebrosides B (**2**) showed much lower mortality than other compounds. Therefore, except compound **1** and **2**, these compounds were introduced as alternatives to synthetic chemical insecticides. However, more research on the purification and characterization of bioactive compounds from entomopathogenic fungi is needed to be compared against *B. tabaci* and other agricultural insect pests in the future.

Declarations

Author Contributions: Conceptualization, R.A. and M.A.; methodology, R.A.; software, M.A.; validation, H.L., Y.W. and A.Z.; formal analysis, R.A. and M.A.; investigation, Y.B.; resources, Y.W.; writing—original draft preparation, R.A. and M.A.; writing—review and editing, Z.Y.; supervision, Y.B. and Z.Y.

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Conflicts of Interest: The authors declare no conflict of interests.

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Figures

Figure 1

The chemical structures of the compounds 1-10

Figure 2

The key COSY and HMBC (arrows) correlations of compound 1

Figure 3

The mean mortality rate of compound 1 and 2 with different concentrations at 24 h, 48 h and 72 h.

Figure 4

The mean morality rate of compound 3 and 4 with different concentrations at 24 h, 48 h and 72 h.

Figure 5

The mean morality rate of compound 5, 6 and 7 with different concentrations at 24 h, 48 h and 72 h.

Figure 6

The mean morality rate of compound 8, 9 and 10 with different concentrations at 24 h, 48 h and 72 h.

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