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Bazzania spiralis Extracts Exhibit Effective Toxicity and Oviposition Deterrence against *Bemisia tabaci*

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ABSTRACT

The silverleaf whitefly, *Bemisia tabaci*, is a major agricultural pest that has developed resistance to many synthetic pesticides, necessitating the search for effective, eco-friendly alternatives. This study investigated the insecticidal potential of crude methanol extracts from the liverwort *Bazzania spiralis* against *B. tabaci*. Toxicity assays demonstrated 100% mortality achieved at 1000µg/ml after 48 hours of exposure. The LC_{50} values ranged from 699.37µg/ml (12 hours) to 22.00µg/ml (60 hours). The extracts exhibited strong oviposition deterrence (31.5%) and reduced egg hatchability (39.39%) at 500µg/ml. Gas chromatography-mass spectrometry analysis revealed eleven compounds in the extract, with sesquiterpenes (59%) and fatty acids (35.4%) as major constituents. The predominant compounds were spathulenol (48.7%) and palmitic acid ethyl ester (22.2%). These findings suggest that *Bazzania spiralis* extracts have potential as a natural alternative to synthetic pesticides for *B. tabaci* management. Further research is needed to isolate active compounds, evaluate field efficacy, and assess environmental persistence and effects on beneficial insects.

Keywords: Biopesticide, GC-MS, insecticidal activity, liverwort, whitefly

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INTRODUCTION

The silverleaf whitefly (*Bemisia tabaci*) is a major pest of chili plants in Malaysia. Whitefly nymphs and adults damage the plants by sucking the sap from leaves,

causing physiological disorders in plants such as chlorotic spots on leaves and abortion of immature fruits. Moreover, the feeding process by adult whiteflies may transmit the lethal begomovirus from one plant to another (Czosnek et al., 2017). The begomovirus-type pepper yellow leaf curl virus (PepYLCV) has been reported to infect chili plants in Indonesia, Thailand and Malaysia, which then exhibit symptoms of leaf deformity and yellowing, resulting in the yield loss of chili production (Fadhila et al., 2020; Laprom et al., 2019; Sau et al., 2020).

Systemic insecticides are preferred for most farmers as they have shown immediate effects against *B. tabaci* and other plant-sucking pests. Carbaryl, malathion and imidacloprid are the world's most utilized and effective pesticides against *B. tabaci* (Abubakar et al., 2022). Humans exposed to imidacloprid have a high likelihood of developing cancer (Caron-Beaudoin et al., 2016). Meanwhile, other neonicotinoid pesticides have been associated with negative effects on non-target organisms, such as honey bees (Mengoni Goñalons & Farina, 2018), vertebrates (Hallmann et al., 2014) and invertebrates (Berheim et al., 2019). A few studies indicate that several biotypes of *B. tabaci* are developing resistance to chemical insecticides. For instance, the Q biotype exhibited significant resistance to commercial insecticides, as observed in a population isolated from Cameron Highland, Pahang, Malaysia (Shadmany et al., 2015).

Terpenes, phenolic and fatty acids are associated with repellent properties to *B. tabaci* (Islam et al., 2017; Yang et al., 2010). Phytochemical investigations on bryophytes have resulted in the isolation of a wide variety of secondary metabolites, namely green leaf volatiles, flavonoids, terpenoids and phenolic compounds (Asakawa et al., 2013; Ludwiczuk & Asakawa, 2019), indicating that these compounds can promote and expand their use as biological insecticides. Among bryophytes, liverworts possess specialized organelles known as oil bodies, a unique structure absent in other bryophytes. The accumulation of secondary metabolites, such as terpenoids and benzenoids, in the oil bodies has been reported (Tanaka et al., 2016). Polygodial sesquiterpenes isolated from *Porella vernicosa* killed mosquito larvae at a concentration of 40 ppm, which was stronger than commercial insect-repellent diethyltoluamide (DEET; Asakawa & Ludwiczuk, 2018). Clavigerins sesquiterpenes from the *Lepidolaena clavigera* have similar efficiency to the active ingredient in pesticide, azadirachtin, against *Anthrenocerus australis* and *Tineola bisselliella* (Perry et al., 2008). It suggests that the production of secondary metabolites in liverworts may have significant implications for plant-herbivore interactions.

Bazzania is the largest genus of the Lepidoziaceae family. Malaysia has documented approximately 66 *Bazzania* species, with 33 identified in Peninsular Malaysia (Lee et al., 2022). Most *Bazzania* sp. are composed of sesquiterpenoid and aromatic compounds (Asakawa et al., 2013). The chemical compositions of Malaysian *Bazzania*, *Bazzania spiralis*, *Bazzania praerupta*, and *Bazzania harpago* have been identified (Ludwiczuk &

Asakawa, 2010). Only *B. harpago* extracts have been tested for antifungal activity (Ng et al., 2021). While in vitro culture has been reported for *Marchantia* sp., similar techniques could potentially be applied to liverworts like *B. spiralis*, offering advantages for biopesticide development due to their ease of culture, rapid life cycles, and ability to establish genetically homogeneous lines through asexual reproduction (Ishizaki et al., 2015; Krishnan et al., 2015). Moreover, their promising terpene profiles make them potential alternatives to chemical pesticides. To investigate the potential of *Bazzania* extracts further, this study aimed to evaluate the insecticidal effects of crude methanol extracts of *B. spiralis* against *B. tabaci* through toxicity and oviposition deterrent assays conducted in a laboratory.

MATERIALS AND METHODS

Sampling Collection

The liverwort species, *Bazzania spiralis*, was collected from Mossy Forest in Cameron Highlands between 8:00 am and 10:00 am in November 2020. The sampling site was located at coordinates 4°31.459'N 101°23.340'E, at 1602 m alt. The recorded temperature was between 15°C and 18°C. The tools and techniques employed for liverwort sample collection in the field adhere to the guidelines provided by Lee and Gradstein (2021). The voucher specimens were identified and deposited in the Herbarium of Universiti Malaysia Terengganu (UMTP). The samples were identified based on morphological characteristics using identification keys (Lee & Gradstein, 2021).

Preparation of Crude Extracts

The preparation of crude extracts was carried out in accordance with Nagappan et al. (2019). The sample was cleaned to remove the substrate and other impurities prior to being air-dried. The air-dried samples were powdered and soaked in 500 ml 80% methanol (Merck, Germany) for four days. The methanol-liverwort mixtures were then filtered using Whatman No.1 (Merck, Germany) filter paper to remove the liverwort remainder, and the filtrate was collected to be concentrated. About 5 g of sodium sulfate anhydrous (Na_2SO_4, Merck, Germany) was added to remove moisture into the filtrate and was left for 30 min at room temperature. The solvent was then filtered and transferred into a round-bottom flask to be concentrated using a rotary evaporator (BUCHI V-700, Switzerland) to obtain the crude extract. The rotary evaporator was set to 25°C, 500-100 mbar, and 125-80 rpm. The pressure and rotation were adjusted within this range to prevent the sample solution from overheating and bubbling. The evaporated extract was transferred into a 20 ml vial and placed in a desiccator to dry out completely. The weight of the dried extract was recorded. Finally, the crude extracts were stored in a -20^{0}C freezer prior to being used for plant treatment and GC-MS analysis.

Bemisia tabaci Rearing

Colonies of *Bemisia tabaci* were collected from *Capsicum annuum* at Kompleks Pertanian UMT Bukit Kor, Terengganu. Colonies of *B. tabaci* were established and maintained on plants in insect-proof cages in a greenhouse at 28°C–32°C and 70%–80% relative humidity. Prior to conducting the toxicity and oviposition assays, newly emerged adults of *B. tabaci* were collected and immobilized by placing them in a refrigerator at 4°C for 2 to 3 minutes. Their sex was distinguished under a stereomicroscope. The females of *B. tabaci* have rounded abdomens, while the males have pointed abdomens. The insects were then placed in flasks and starved for two hours prior to the experiments.

Toxicity Assay

The crude extract was dissolved in absolute ethanol (Merck, Germany) to prepare stock solutions with a 10 mg/ml concentration. The stock solutions were then diluted with a series of concentrations of (1000µg/ml, 500µg/ml, 250 µg/ ml, 125 µg/ml, and 62.5 µg/ ml) and mixed with 0.02% (v/v) Tween 20 (Thermo Scientific, USA) as a surfactant. As for the negative control, a mock solution consisting of a mixture of 70% ethanol and 0.02% (v/v) Tween 20 was prepared. A commercial pesticide, Imidacloprid (Fusilier, Malaysia), was prepared according to the manufacturer's recommended doses and mixed with 0.02% of Tween 20.

This experiment was conducted by following Chen et al. (2018) with modifications. A young, fully extended leaf from a *Capsicum annuum* (variety Kulai 461) plant was sprayed with 1 ml of liverwort crude extracts at various concentrations and was left to dry for 12 hours. The leaf was placed on fresh agar inside a round plastic container (25oz) with a 1-inch agar layer to prevent desiccation. Ten adult insects (five females and five males) were placed into each container. The number of dead and surviving *B. tabaci* individuals was observed under a stereomicroscope after 48 hours. *Bemisia tabaci* was considered dead when no movements of antennae or legs were observed. For the negative control, a leaf was sprayed with a mock solution before infestation. Three leaves per plant and five *C. annuum* plants were used for this experiment. The assay was carried out in a chamber with a temperature of 24°C–26°C, relative humidity of 60%–70%, and a photoperiod of 12-h lights. The mortality rates of *B. tabachi* were compared between different concentrations of liverwort extract, pesticide and negative control.

Oviposition Deterrent Bioassay

To investigate the effect of liverwort crude extract on *B. tabaci*, an oviposition deterrent assay was conducted following Saad et al. (2017). 1 ml of 500µg/ml of liverwort extract solution was sprayed onto leaves of *C. annuum* plants, and then left for 24 hours. Ten adult males and 10 adult females of *B. tabaci* were collected from a rearing cage and placed in a glass jar containing agar and fresh leaves for 24 hours for copulation. Three leaves

from nodes 12 to 14 of each *C. annuum* plant were covered with an insect-proof clip cage (30 mm diameter). Three female adults of *B. tabaci* from the glass jar were subsequently placed in each clip caged. The *B. tabaci* were allowed to feed and oviposit for two days. The number of eggs laid was counted, and the adults were removed from the leaves. On the seventh day, the leaves were detached, and the number of larvae was counted. For the negative control, each leaf was sprayed with a mock solution. The *C. annuum* leaves were also sprayed with a synthetic pesticide, an Imidacloprid solution, as positive control. Five plants were used for the experiment, with each plant having three clip cages per treatment and placed in a separate insect cage (24°C–26°C, 60%–70% RH, 12:12-h lights: dark). The number of survival larvae was considered as viable eggs. The egg hatching rate was determined by the number of viable eggs relative to the total number of laid eggs and was expressed in percentage. An oviposition deterrent index (ODI) was calculated using the formula:

$$T = 100\ (C - T)/\ (C + T)$$

Where C is the total number of eggs laid on control leaves, and T is the total number of eggs on treated leaves (Huang et al., 1994).

Data Analysis

The percentage of mortality was corrected using Abbott's formula (Abbott, 1925). The lethal concentrations (LC_{50} and LC_{90}) and lethal times (LT_{50} and LT_{90}) were calculated using probit analysis. The mortality data were analyzed using the Kruskal-Walis H-test to calculate mean differences between concentrations, followed by post-hoc Dunn's test (*p*-values corrected according to the Bonferroni method for multiple comparisons). Mean differences among plant treatments for oviposition and hatchling rate were analyzed independently using one-way analysis of variance (ANOVA), followed by post hoc Tukey's test. All statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) version 29.0 software.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

Approximately 1 mg of crude extracts of *B. spiralis* were dissolved in 1 ml of methanol (GC grade, Sigma-Aldrich, Germany) and filtered using a 0.45 µM syringe filter. The GC-MS was performed using SHIMADZU QP2010 Ultra gas chromatograph–mass spectrometer (Japan) equipped with a Zebron ZB-5ms column (30 m × 250 µm i.d. × 0.25 µm film thickness; Phenomenex, USA). The gas chromatographic parameters were as follows: the initial temperature was fixed at 50°C for 1 min, then increased at a rate of 10 °C min^{-1} to 200°C, then further increased to 300°C at 5°C min^{-1}. The injection temperature was set at 300°C, and an injection volume of 1 µL was used in splitless mode. The MS scan range

was set at 50–600 m/z at 70 eV. Helium was used as the carrier gas at a 1 mL/min flow rate. Before GC-MS analysis, the n-alkane standard, C_7-C_{30} (Sigma-Aldrich, Germany), was used and run using the same parameters described above.

Compounds were identified by comparing the GC-MS mass spectra with NIST library spectra. The retention indices were calculated using the method described in Van Den Dool and Kratz (1963). Experimental retention indices (RI) were compared with reported RI in literature (Babushok et al., 2011) or in NIST (https://webbook.nist.gov/chemistry/). The volatile composition was expressed as the percentage of peak area relative to the total peak area of each compound.

RESULTS

Toxicity and Oviposition Deterrent to *Bemisia tabaci*

The liverworts' crude extracts were tested for toxicity against adult *B. tabaci*. The results showed that *B. tabaci* mortality was recorded as 58.3 ± 3.7% at a concentration of 1000 µg/ml after 12 hours of exposure and reached 100% after 48 hours (Figure 1). The crude extracts of *B. spiralis* showed maximum mortality on *B. tabaci* at concentrations of 500µg/ml and 250 µg/ml after 60 hours (Kruskal-Wallis test: H_6=31.83, p<0.001). After 60 hours of exposure, mortality was recorded as 91.7 ± 3.7% and 86.1 ± 5.1% at 125µg/ml concentrations and 62.5µg/ml, respectively. The mock treatment showed the lowest mortality compared to *Bazzania*-treated leaves, while imidacloprid showed maximum mortality after 24 hours of exposure.

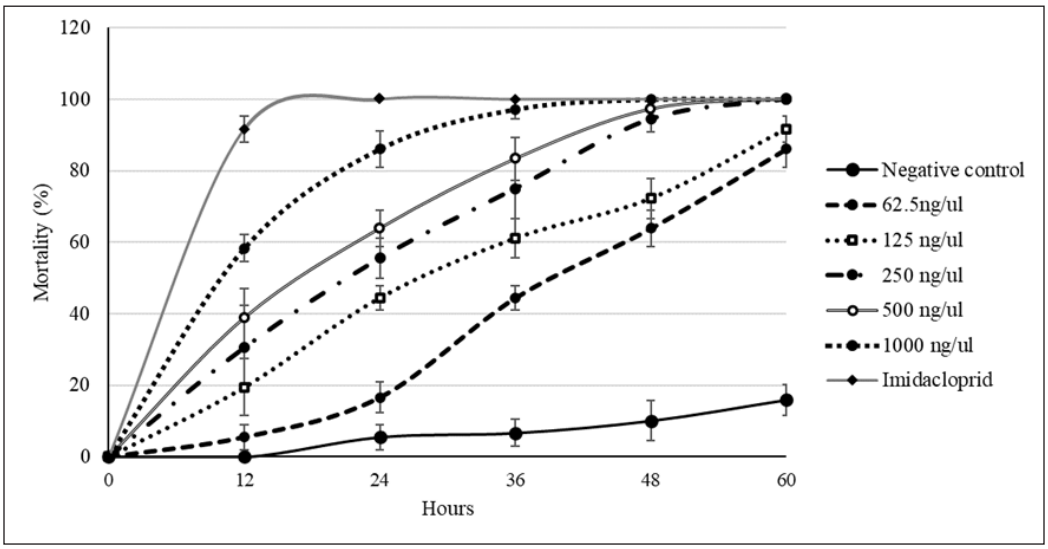

Figure 1. Accumulated mortality of *B. tabaci* in a glass jar exposed to different concentrations of *Bazzania spiralis* crude extracts. The data represents the mean of six replicates for each concentration
Note. Vertical bars represent the standard error (SE) of the mean

The LC_{50} values indicated that *B. tabaci* is sensitive to the *B. spiralis* crude extract, ranging from 699.37µg/ml after 12 hours of exposure to 22.00µg/ml after 60 hours (Table 1). The corresponding LC_{90} values were estimated to be 84.88µg/ml after 60 60-hour exposure period of *B. tabaci* to *B. spiralis* crude extracts. The estimated values of LT_{50} ranged from 10.91 hours to 40.15 hours, while LT_{90} values occurred from 24.25 hours to 78.07 hours at concentrations of 1000µg/ml to 62.5 µg/ml (Table 2).

Table 1
Toxicity of B. spiralis *crude extracts to* B. tabaci *after different exposure time*s (*N*=36)

Exposure time (hours)	**LC_{50} (95% CL)[c] (ug/ml) [a]**	**LC_{90} (95% CL)[c] (ug/ml) [b]**	**Slope ± SE**	x^2
12	699.37 (473.81-1371.25)	6512.82 (2647.33-9914.28)	1.32 ± 0.26	0.96
24	214.30 (152.36-292.83)	1570.76 (917.09-3273.55)	1.48 ± 0.25	2.45
36	81.94 (43.35-118.90)	622.95 (401.84-1423.20)	1.46 ± 0.28	1.14
48	47.11 (20.75-70.07)	215.91 (157.05-370.42)	1.94 ± 0.41	1.95
60	22.00 (6.24-43.82)	84.88 (40.67-141.08)	2.19 ± 0.84	1.44

Notes.
[a] Lethal concentration required to kill 50% of *B. tabaci*
[b] Lethal concentration required to kill 90% of *B. tabaci*
[c] Confidence limit, which was calculated with 95% confidence

Table 2
Toxicity of B. spiralis *crude extracts to* B. tabaci *after different exposure concentrations (N=36)*

Concentration (µg/ml)	**LT_{50} (95% CL)[c] (hours) [a]**	**LT_{90} (95% CL)[c] (hours) [b]**	**Slope ± SE**	x^2
62.50	40.15 (35.51-46.12)	78.07 (63.97-108.49)	4.00 ± 0.57	3.35
125.00	28.98 (24.21-34.24)	74.28 (57.71-114.81)	2.82 ± 0.44	2.35
250.00	19.14 (15.54-22.37)	45.15 (37.67-59.32)	3.44 ± 0.49	5.24
500.00	16.18 (15.59-15.25)	38.17 (32.37-50.94)	3.37 ± 0.58	3.92
1000.00	10.91 (7.38-13.57)	24.25 (20.14-31.81)	3.70 ± 0.70	1.07

Notes.
[a] Lethal time required to kill 50% of *B. tabaci*
[b] Lethal time required to kill 90% of *B. tabaci*
[c] Confidence limit, which was calculated with 95% confidence

The crude extracts of *B. spiralis* strongly deterred oviposition by *B. tabaci*, with a significantly lower number of eggs being laid on *B. spiralis*-treated leaves in comparison with the control ($F_{2,12}$=47.01, p<0.001) (Figure 2). The oviposition deterrent activity of *B. spiralis* extracts was recorded as 31.5% ± 10.44 at 500µg/ml (Table 3). The positive control, imidacloprid, demonstrated a 2-fold higher oviposition deterrent activity than *B. spiralis* extracts. The number of eggs hatching on *B. spiralis*-treated leaves was significantly lower than the control at a concentration of 500 µg/ml after seven days of treatment (Figure 2). The crude extracts of *B. spiralis* reduced the *B. tabaci* eggs' hatchability to 39.39%±4.56 ($F_{2,12}$=200.09, p<0.001).

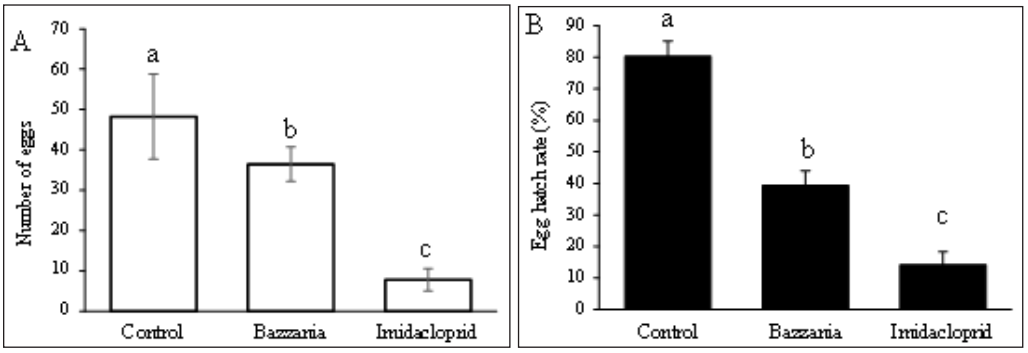

Figure 2. Effects of liverwort extracts (500µg/ml) on oviposition (A) and egg hatching (B) of *B. tabaci*. Mock is the negative control, and imidacloprid is the synthetic pesticide. Different letters indicate significant differences among treatments (ANOVA followed by Tukey's post hoc analysis, p<0.05)

Table 3
The oviposition deterrent effects of liverwort crude extracts on Bemisia tabaci

Liverwort species	Egg number in treated leaves	Egg number in control leaves	Effective deterrence (%)	Egg number in imidacloprid leaves	Effective deterrence (%)
Bazzania spiralis	53 ± 7.44	36.4 ± 4.28	31.5 ± 10.44	7.8 ± 2.77	83.78 ± 7.48

Gas Chromatography-Mass Spectrophotometry (GC-MS) Analysis

The crude extract of *B. spiralis* revealed the presence of eleven compounds, corresponding to 97.8% of the total extract (Table 4). Among these compounds, 64% were fatty acids, representing 35.4% of the total peak area. Despite having the highest number of fatty acids derivatives, sesquiterpene recorded greater peak areas, constituting 59% of the total area. spathulenol exhibited the highest peak area (49.8%), followed by palmitic acid ethyl ester (22.7%).

Table 4
Chemical composition of the crude extracts from Bazzania spiralis

No.	Compounds	RT	RI	RI_{ref}	Peak area (%)
1	Ledene	14.17	1497	1494	2.9
2	Spathulenol	15.57	1608	1605	48.7
3	Aristolone	17.28	1758	1757	2.8
4	Hexahydrofarnesyl acetone	18.21	1843	1843	3.3
5	Palmitic acid, methyl ester	19.07	1914	1920	trace
6	Palmitic acid, ethyl ester	19.94	1960	1976	22.2
7	(9Z,12Z)-9,12-Octadecadien-1-ol	21.33	2067	2069	3.2
8	Trichloroacetic acid, tridec-2-ynyl ester	21.39	2073	2075	8.3
9	cis-10-Heptadecenoic acid	21.45	2080	2084	2.3
10	Heptadecanoic acid, ethyl ester	21.63	2099	2089	1.7
11	Phthalic acid, 2-ethylhexyl tetradecyl ester	24.49	2429	2475	trace
12	Stigmasterol	30.71	3175	3170	2.3
				Total	97.8

Note. RI = Retention indices relative to n-alkanes (C_7-C_{30}). $RI_{ref:}$ Retention indices with those reported in references

DISCUSSION

The present study demonstrates the insecticidal potential of *Bazzania spiralis* crude extracts against the whitefly *Bemisia tabaci*. The extracts exhibited significant toxicity, oviposition deterrence, and egg hatchability reduction, suggesting their potential as a natural alternative to synthetic pesticides for the management of *B. tabaci*. Asakawa et al. (2013) reported that several liverwort species, including the genus *Bazzania*, contain bioactive compounds with insecticidal activities.

To date, no study has reported the toxicity of *Bazzania* sp. extracts against various insect pests. This study showed that the mortality of *B. tabaci* reached 100% at a concentration of 1000 ug/ml after 48 hours of exposure, indicating the potent insecticidal activity of the extracts. Similar studies have reported the toxicity of liverwort extracts against various insect pests. For instance, Mulyani et al. (2024) found that crude extracts of the liverwort *Marchantia paleacea* showed high toxicity against the larvae *Athalia proxima*, with an LC_{50} value of 0.33% after 24 hours of exposure. The LC_{50} values of *B. spiralis* extracts ranged from 699.37µg/ml (12 hours of exposure) to 22.00µg/ml (60 hours of exposure), suggesting that prolonged exposure to lower concentrations can still achieve significant mortality. These findings are consistent with the LC_{50} values of the liverwort *Plagiochila asplenioides* extracts against the diamondback moth, *Plutella xylostella*, which decreased from 245.7 µg/ml (24 hours of exposure) to 76.3 µg/ml (72 hours of exposure) (Zhang et

al., 2020). *Bazzania spiralis* crude extracts significantly deterred oviposition and reduced egg hatchability, demonstrating their potential as oviposition deterrents and ovicides against *B. tabaci*. Similar findings have been reported that crude extracts of the liverwort *Marchantia linearis* exhibited oviposition deterrent activity against the *Spodoptera litura* (Krishnan & Kumara, 2015).

Neonicotinoid pesticides, such as imidacloprid, are highly effective and long-lasting due to their systemic nature. These water-soluble compounds are easily absorbed by plant tissues and transported throughout the plant, providing extended protection against pests like whiteflies and aphids. Imidacloprid affects the central nervous system of insects, leading to paralysis and death. However, it also poses similar risks to beneficial insects, particularly pollinators, contributing to the decline of pollinator populations (Ihara & Matsuda, 2018). Recent studies have shown that imidacloprid triggers a significant oxidative stress response in *B. tabaci*, producing extensive ROS (Li et al., 2024). Our study demonstrates that *B. spiralis* crude extracts achieve 90% mortality of *B. tabaci* within 24 hours of application, suggesting a potential to exhibit similar effects due to the significant increase in *B. tabaci* mortality. Both imidacloprid and *B. spiralis* extracts induce trichome formation in treated plants (unpublished data), indicating absorption into plant tissues. Botanical pesticides like *B. spiralis* extracts typically degrade more quickly than synthetic systemic insecticides (Khursheed et al., 2022). This rapid degradation reduces the risk of toxin accumulation during flowering periods, potentially mitigating harmful effects on beneficial insects. These characteristics suggest that *B. spiralis* extracts could offer a more environmentally friendly alternative to synthetic pesticides, potentially reducing the risk of resistance development and negative impacts on non-target species.

GC-MS analysis of *B. spiralis* crude extracts revealed the presence of eleven compounds, with fatty acids derivatives and sesquiterpenes being the major constituents. Spathulenol, composed of the highest composition of the total compounds in *B. spiralis* extracts, is also isolated from *B. spiralis* and *B. praerupta* (Ludwiczuk & Asakawa, 2010). Diethyl ether extracts of *B. spiralis* from Mount Kinabalu, Sabah, have identified a tetracyclic triterpene, stigmasterol (Kondo et al., 1990), which was also detected in GC-MS of methanolic extracts in this study. Spathulenol has the potential to contribute to the toxicity and inhibition of *B. tabaci*. As a sesquiterpene, spathulenol is lipophilic and volatile, allowing it to penetrate insects rapidly and interfere with their physiological functions (Albouchi et al., 2018; Bakkali et al., 2008). It has been shown to exhibit strong antifeedant activity against phloem feeders, green peach aphids, *Myzus persicae* (Souda et al., 2023), and exhibit neurotoxic effects on the grain aphid, *Metopolophium dirhodum* (Benelli et al., 2020). However, the exact mechanisms by which spathulenol affects *B. tabaci* in our study are not fully understood and merit further investigation.

Palmitic acid, another major component identified in the extracts, has demonstrated significant effects on various insect pests. Palmitic acid showed nymph toxicity and oviposition deterrence against *B. tabaci*, resulting in 55% mortality and 40.5% deterrence in a 24-hour bioassay (Wagan et al., 2018). These effects may be related to palmitic acid's ability to modulate the synthesis and release of insect hormones, such as juvenile hormones and ecdysteroids (Stanley-Samuelson et al., 1988). Disruption of these hormones can lead to developmental abnormalities, reduced fecundity, and increased mortality (Singtripop et al., 2000). The mechanism of action for palmitic acid may also involve neurological effects. Research on the light brown apple moth, *Epiphyas postvittana*, has shown that fatty acids with carbon chains C_{14} to C_{16} can increase the permeability of neuron membranes, affecting ion exchange and provoking stressed excitability of neuron cells (Taverner et al., 2001). Similarly, *Tetranychus cinnabarinus* exposed to palmitate exhibited signs of neural poisoning (Wang et al., 2009). While palmitic acid falls within this category, further research is needed to determine if it acts similarly in *B. tabaci* when exposed to *B. spiralis* extracts.

The presence of fatty acids and sesquiterpenes in *B. spiralis* crude extracts indicates the potential for synergistic effects. Synergistic toxicity commonly occurs among compounds found in essential oils (Pavela, 2014), as well as among the major constituents of these oils in their natural proportions (Hummelbrunner & Isman, 2001). For instance, sesquiterpenes facilitate the penetration or transport of another compound across the insect cuticle or gut membrane (Tak & Isman, 2017). They may enhance the penetration of fatty acids to reach the target sites. Synergistic interactions can also influence changes in insect behavior. One compound may act as a feeding or oviposition deterrent, while another may exhibit toxic effects. The combination of these effects can lead to reduced feeding damage and population growth (Miresmailli & Isman, 2014). The synergistic action of sesquiterpenes and fatty acids in *B. spiralis* extracts may enhance their insecticidal efficacy against *B. tabaci*.

For practical field application of *B. spiralis* extracts, we recommend exploring fumigation methods in greenhouse settings and contact spraying for open-field use. However, the extract's stability under various environmental conditions requires further investigation. Comprehensive field trials are necessary to assess the extract's efficacy and persistence under diverse environmental conditions, which will be crucial for optimizing application strategies.

CONCLUSION

This study demonstrates the insecticidal properties of *B. spiralis* crude extracts against *B. tabaci*, including toxicity, oviposition deterrent activity, and reduction in egg hatchability. Sesquiterpenes, particularly spathulenol, and fatty acids in the extracts may contribute to their insecticidal activity. Moreover, the potential synergistic effects between these

sesquiterpenes and fatty acids could enhance the overall effectiveness of the extracts, highlighting their complexity as a natural insecticidal agent. These findings highlight the potential of liverwort-derived compounds as natural alternatives to synthetic pesticides for managing *B. tabaci*. Further research is needed to isolate and characterize the active compounds responsible for the insecticidal properties, investigate their potential synergistic interactions, and evaluate their efficacy under field conditions.

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