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Research Article

Chemical Constituents of *Plectranthus tomentosa* Extract and Its Control Effect on *Tetranychus kanzawai*

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The repellent and contact effects of *Plectranthus tomentosa* extracts of leaf and stem tissue on different stages of *Tetranychus kanzawai* were tested using the leaf-dip and insect-dip methods, and the constituents of the *Plectranthus tomentosa* extract were also determined. It was found that the repellent effect on *Tetranychus kanzawai* female adults has reached up to 84.43% after 60 min, with significant mortality of *Tetranychus kanzawai* at all growth stages. Sixty-nine components of the extract were identified by gas chromatography-mass spectrometry, of which limonene, followed by terpinolene, were present at the highest concentration. This research demonstrated that *Plectranthus tomentosa* has strong repellent and contact effects against *Tetranychus kanzawai*, offering potential natural strategies for the environmentally protective control of *Tetranychus kanzawai* in tea gardens and providing the foundation for the biomimetic synthesis of pesticides.

1. Introduction

In the era of "green food," "green agriculture," "sustainable agriculture," and integrated pest management (IPM), environmental standards of pesticides are becoming more and more stringent. Extracting substances with insecticidal activity from plants or directly processing them into new botanical pesticides and using them as leading compounds to synthesize new, safer, and more efficient pesticides have become hot topics of research.

Tetranychus kanzawai is one of the major pest mites that harm tea trees, strawberries, and vegetables, exploring pollution-free control methods to control the pest and is necessary to produce relevant green agricultural products. Botanical acaricides are of great interest recently, as they represent an important method for pollution-free control of mites. Grange and Ahmed have reported about 2400 species of plants with pest control activity early in 1988 [1]. Until now, the most studied acaricidal plants mainly belong to the Daphneaceae, Meliaceae, Solanaceae, Leguminosae, and Compositae families. Huang et al. screened acetone extracts of 121 species of plants in 51 families and 94 genera collected from Qinling Mountains and Inner Mongolia for aphidicidal and acaricidal activities [2]. Huo screened 117 species of plants in northwest China for acaricidal activity and found that acetone extracts of 71 species had good acaricidal activity against *Tetranychina harti* [3]. Jia et al. studied the biological activity of extracts of 8 indoor plants against *Tetranychus cinnabarinus* [4]. Liang et al. and Zhang et al. studied acaricidal substances of *Stellera chamaejasme* L. and found that daphnetin, daphnetin, squalene, β-sitosterol, and scopolactone were the key constituents responsible for the acaricidal activity [5–8]. Zhao et al. studied the components in volatile oil of hops and their acaricidal activity [9]. Zhou et al. studied the biological activity and mode of action of scoparone on different mite states of *Tetranychus cinnabarinus* [10]. Hu et al. studied the synergistic effect of abamectin and plant essential oils to kill *Tetranychus cinnabarinus* [11]. Onder et al. had proved that the essential oils of *Micromeria fruticosa* L., *Nepeta racemosa* L., and *Origanum vulgare* L. had biological activity against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn [12]. Cavalcanti et al. measured the acaricidal effect of 20 chemical components in the plant essential oil of *Lippia sidoides* chain, and the results showed that thymol and carvacrol had a fumigation effect on *Tetranychus urticae* Koch [13]. Zou et al. found curcumin 2,4-dinitrophenylhydrazine derivative showed a good control effect on *Tetranychus cinnabarinus* [14]. As for *Plectranthus tomentosa*, Zhao et al. and Meng et al. had explored the bacteriostatic effect of *Plectranthus tomentosa* [15, 16], and Xiong et al. had identified its volatile components [17]; however, there is no research study about the insecticidal or acaricidal activity of *Plectranthus tomentosa*. On the other hand, research on pollution-free control of *Tetranychus kanzawai* had been focused on predatory natural enemies of mites [18–22], while botanical pesticides for this pest have not been well studied.

In this study, the repellent and contact effect of *Plectranthus tomentosa* on *Tetranychus kanzawai* and the constituents of *Plectranthus tomentosa* extract were determined by GC-MS to provide references for the development of botanical acaricide and methods for pollution-free control of tea garden mites.

2. Materials and Methods

2.1. Sources of Test Insect and Plant. *Tetranychus kanzawai* was collected from the tea gardens in Huachu town, Puding County, Anshun City, Guizhou Province, and has been bred with strawberry leaves in the laboratory of Anshun College for more than three generations before use. After that, the adults, larvae, and eggs of *Tetranychus kanzawai* were produced according to the method introduced by IRAC (Insecticidal Resistance Action Committee, 2000). Fresh strawberry leaves were collected, washed, briefly dried, and placed in a humidified dish padded with a wet sponge and plastic wrap, with the petioles wrapped with wet cotton. Thirty female adult mites were released to the area with a #0 soft brush, and the dish was placed in the insect breeding room to allow the mites to lay eggs for 12 h; then, the adult mites were removed. The worms hatched from the eggs on the leaves were in the same worm state, so adult mites, larvae, and eggs at the same growth stage can be produced.

Plectranthus tomentosa was collected from the greenhouse of Anshun Academy of Agricultural Sciences. The plant was artificially propagated and cultivated to about 10 cm in height in Anshun College. The flesh stems and leaves were harvested and shattered with a multifunction food blender into a paste. The filtrate was centrifuged at 4000 rpm for 5 min at 20°C, and the *Plectranthus tomentosa* fresh juice was collected and stored in the refrigerator until use. While, the fully dried branches and leaves of *Plectranthus tomentosa* were pulverized into powder and filtered with an 80-mesh sieve. Fifty grams of the dry powder and 500 mL of acetone were added to a conical flask and irradiated with a microwave oven at 400 W power for 50 s. The mixture was filtered to get the solution, and the residue was reextracted twice with the same method. The filtrates were combined and concentrated under reduced pressure with a rotary evaporator at 40°C till the organic solvent was completely evaporated, and the extract was stored at 4°C for later use.

Table 1: Repellent rate of *Plectranthus tomentosa* fresh juice on *Tetranychus kanzawai*.

Time (min)	Repellent rate (%) (mean ± SE)*
5	72.23 ± 5.08^{bc}
30	81.10 ± 1.91^{ab}
60	84.43 ± 1.96^{a}

*Different lowercase letters in the same column revealed that the difference is significant with $p < 0.05$.

2.2. Experimental Method

2.2.1. Repelling Effect on Female Adult Mites of Tetranychus kanzawai Fresh Juice. The repellent effect of *Plectranthus tomentosa* fresh juice on *Tetranychus kanzawai* was tested by using the leaf-dipping method. Briefly, filter paper with a diameter of 9 cm was cut in half along the midline, one half was soaked in 10 mL 10% *Plectranthus tomentosa* fresh juice diluted with pure water, and the other half was soaked in 10 mL pure water served as a control. Both halves were naturally dried, stuck together along the cut with transparent tape, and fixed to the bottom of a clear Petri dish (9.0 cm × 2.0 cm) with double-sided tape. Vaseline was applied around the filter paper to prevent the adult mites from escaping. Thirty adult female mites were placed at the junction of the juice-soaked area and the control area. After 15, 30, and 60 min, the distribution of female adult mites on the two sides was recorded, and the repellent rate was calculated.

$$\text{Repellent rate} = \frac{(\text{number of test mites in the control area} - \text{number of test mites in the tjuice} - \text{soaked area})}{\text{number of test mites in the control area}} \times 100\%. \quad (1)$$

2.2.2. The Contact Effect of the Extract of Plectranthus tomentosa on Tetranychus kanzawai. The contact effect of the extract of *Plectranthus tomentosa* on *Tetranychus kanzawai* was determined using the insect-dipping method. Briefly, the *Plectranthus tomentosa* extract was dissolved and diluted with 0.1% Tween-80 aqueous solution to prepare 1000, 100, and 50 mg/mL concentrations. Thirty adult mites at the same growth stage on the same tea leave were immersed into the extract solution for 5 s and then naturally dried and put back into the humidified dish in an incubator with 25 ± 1°C, 60%–80% humidity, and a 16/8 light/dark cycle. For adult and larvae mites, the total number and death number were recorded to calculate the survival rate, and the hatching rate was calculated for eggs. Three duplicates were set for each test, and a 0.1% Tween-80 aqueous solution served as a control.

2.2.3. Determination of the Constituents in Plectranthus tomentosa Extract. *Plectranthus tomentosa* extract was

Table 2: The contact effect of *Plectranthus tomentosa* extract on *Tetranychus kanzawai*.

Concentration (mg/mL)	Mortality rate (%) (mean ± SE)*				
	Female adult		Larval		Eggs
	24 h	48 h	24 h	48 h	
1000	61.11 ± 1.93^{bc}	66.67 ± 3.34^{bc}	64.44 ± 1.93^{bc}	66.67 ± 3.34^{bc}	72.22 ± 2.09^{c}
100	65.56 ± 1.93^{b}	67.78 ± 1.92^{b}	66.67 ± 3.33^{b}	70.00 ± 3.33^{b}	77.78 ± 2.09^{b}
50	77.77 ± 5.09^{a}	83.33 ± 3.34^{a}	82.22 ± 5.09^{a}	86.67 ± 3.34^{a}	85.56 ± 1.92^{a}

*Different lowercase letters in the same column revealed the significant difference with $p < 0.05$.

Table 3: Composition and relative contents of *Plectranthus tomentosa* extract.

No.	Retention time	Compound name	Molecular weight	Relative content
1	7.41	Limonene	136	34.69
2	7.62	2 (3H)-Furanone, 5-ethenyldihydro-5-methyl-	126	0.08
3	8.63	Bicyclo [3.1.0] hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha., 2.beta., 5.alpha.)-	154	0.13
4	9.02	*trans*-p-Mentha-2,8-dienol	152	0.51
5	9.28	*cis*-p-Mentha-2,8-dien-1-ol	152	0.17
6	9.37	Bicyclo [3.1.1] heptan-3-ol, 6,6-dimethyl-2-methylene-	152	0.04
7	9.89	Bicyclo [3.3.0] oct-2-en-7-one, 6-methyl-	136	0.16
8	10.02	Benzene, 1,3-dimethyl-5-(1-methylethyl)-	148	0.06
9	10.27	Terpineol	154	0.14
10	10.59	7-Methyl-1,2,3,5,8,8a-hexahydronaphthalene	148	0.06
11	10.78	*trans*-Carveol	152	0.14
12	10.95	*cis*-p-Mentha-1 (7), 8-dien-2-ol	152	0.52
13	11.28	D-carvone	150	0.07
14	11.82	1,7-Octadiene-3,6-diol, 2,6-dimethyl-	170	0.73
15	12.15	Bornyl acetate	196	2.20
16	12.31	(1S, 2R, 4R, 7R)-4-Isopropyl-7-methyl-3,8-dioxatricyclo [5.1.0.02, 4] octane	168	0.08
17	12.42	Cyclohexasiloxane, dodecamethyl-	444	0.21
18	12.59	alpha.-Cubebene	204	1.07
19	13.00	Terpinolene	136	8.66
20	13.33	alpha.-Copaene	204	0.77
21	13.62	Bicyclosesquiphellandrene	204	4.10
22	13.83	Methyl eugenol	178	1.12
23	14.29	Caryophyllene	204	1.13
24	14.64	alpha.-Guaiene	204	0.08
25	14.94	Valerena-4,7 (11)-diene	204	0.07
26	15.44	Alloaromadendrene	204	0.49
27	16.01	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	202	0.09
28	16.18	(3R, 3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-dimethyloctahydro-1h-cyclopenta [1, 3] cyclopropa [1,2] benzen-3-ol	222	0.77
29	16.37	*trans*-Calamenene	202	0.12
30	16.48	Pacifigorgiol	222	0.12
31	17.30	(1aR, 4S, 7R, 7aS, 7bR)-1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1h-cyclopropa[e]azulen-4-ol	220	0.08
32	18.13	(-)-Spathulenol	220	1.09
33	18.36	(-)-Globulol	222	1.36
34	18.97	(1R,2R,4S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decan-4-ol	220	0.35
35	19.72	Ledol	222	0.76
36	20.02	Muurola-4,10 (14)-dien-1.beta.-ol	220	0.22
37	20.31	2-Naphthalenemethanol, decahydro-.alpha.,.alpha.,4a-trimethyl-8-methylene-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	222	0.52
38	20.44	(1R, 4S)-4-Isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalen-1-ol	218	0.60
39	20.52	1 (2H)-Naphthalenone, octahydro-4a, 8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a.alpha., 7.beta., 8a.alpha.)]-	222	3.28
40	20.81	(1R, 7S, E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	220	0.60
41	21.00	(E)-3-((4S, 7R, 7aR)-3,7-Dimethyl-2,4,5,6,7,7a-hexahydro-1h-inden-4-yl)-2-methylacrylaldehyde	218	1.29
42	21.58	(-)-Aristolene	204	0.86

Table 3: Continued.

No.	Retention time	Compound name	Molecular weight	Relative content
43	21.83	Cycloheptane, 4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-vinyl-	204	0.06
44	22.82	Megastigma-4, 6 (Z),8 (E)-triene	176	0.09
45	22.98	2-Pentadecanone, 6,10,14-trimethyl-	268	0.72
46	23.34	Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl-	188	0.83
47	23.79	Phthalic acid, 7-bromoheptyl butyl ester	398	1.60
48	23.90	1H-Indene-4-acetic acid, 6-(1,1-dimethylethyl)-2,3-dihydro-1,1-dimethyl-	260	0.39
49	24.45	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	270	0.06
50	25.74	Hexadecane, 2,6,10,14-tetramethyl-	282	0.21
51	26.10	Octadecane	254	2.23
52	26.50	Heptadecane	240	2.83
53	26.83	Docosane	310	0.20
54	27.65	1-(2-Methoxyphenyl)-2,5-dihydro-1H-pyrrole-2,5-dione	203	1.29
55	27.82	2-Hydroxy-3,4-tetramethylene-6-methoxy quinoline	229	0.06
56	27.90	Hentriacontane	437	1.92
57	28.19	Ferruginol	286	0.29
58	28.47	3-Bromobenzyl alcohol, TMS derivative	258	0.89
59	28.69	Tetracosane	338	0.48
60	29.19	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-	328	0.18
61	30.10	Aristolene epoxide	220	0.08
62	30.86	(2Z, 4E)-3,7,11-Trimethyl-2,4,10-dodecatriene	206	0.07
63	32.18	Davana ether	234	0.05
64	32.74	Heneicosane	296	4.17
65	33.29	Nonadecane, 9-methyl-	282	0.48
66	33.72	9,10-Dihydrodeoxynivalenol	298	0.05
67	34.15	Heptacosane	380	2.83
68	34.61	Octacosane	394	0.69
69	35.27	Triacontane	422	0.69

redissolved with 2 mL *n*-hexane and filtered with a 0.22 μm organic filter. The constituents in *Plectranthus tomentosa* extract were analyzed using a gas chromatography-mass spectrometer (Agilent GC-MS 7890B 5977A, Agilent Technologies, Palo Alto, CA).

2.3. Statistical Analysis. SPSS 24.0 was used for the statistical analysis of the data. The data of parallel tests were expressed by mean ± standard error ($X \pm$ SE), and Duncan's new complex range test was used for multiple comparisons.

3. Results and Discussion

3.1. Repellent Effect Test of Plectranthus tomentosa Fresh Juice on Tetranychus kanzawai. The results of the repellent effect of the *Plectranthus tomentosa* fresh juice on the female adults of *Tetranychus kanzawai* are given in Table 1. As given in Table 1, *Plectranthus tomentosa* fresh juice showed a good repellent effect on female *Tetranychus kanzawai*. The repellent rate reached 70% early at 15 min and was up to 84% at 60 min. The difference in repellent rate was not statistically significant between 15 min and 30 min or between 30 min and 60 min, but was statistically significant between 15 min and 60 min.

3.2. Contact Effect of Plectranthus tomentosa Extract on Tetranychus kanzawai. The contact effect of *Plectranthus tomentosa* extract on *Tetranychus kanzawai* at different growth stages is given in Table 2. As given in Table 2, *Plectranthus tomentosa* extract at 50 mg/mL killed 77.77% and 83.33% of the female adults at 24 and 48 h, respectively; the mortality rate was significantly higher than that at either 1000 mg/mL or 100 mg/mL. Meanwhile, Table 2 provides that extract at 50 mg/mL revealed the strongest contact effect on the larval of *Tetranychus kanzawai*, with the mortality rate of 82.22% and 86.67%, respectively, which were significantly higher than that at either 1000 mg/mL or 100 mg/mL. In addition, Table 2 provides that the extract at 50 mg/mL showed the strongest effect, killing 85.56% of the eggs, which was significantly higher than that at either 1000 mg/mL or 100 mg/mL, although the two latter concentrations still achieved greater than 72% egg mortality.

3.3. Determination of Chemical Constituents in the Extract. The chemical constituents in the extract were determined using GC-MS and the results are given in Table 3 and Figure 1. Table 3 and Figure 1 show that a total of 69 constituents were identified in the extract, accounting for 93.03% of the total mass. The extract contained 14 alkanes, 14 alkenes, 23 alcohols, 6 aldehydes, 1 ketone, 1 acid, 2 esters, 1 ether, and 7 aromatic hydrocarbons accounting for 17.08%, 52.28%, 10.06%, 5.60%, 1.29%, 0.39%, 3.8%, 0.05%, and 2.48% of the total volatile components, respectively. Limonene was the most abundant single constituent (34.69% of the total), followed by terpinolene (8.66%). It had been

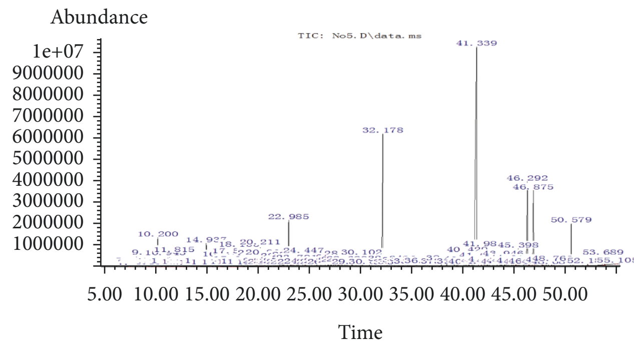

Figure 1: Ion current spectrum of the extract of *Plectranthus tomentosa*.

reported previously that limonene was also the key constituent responsible for the repellent effect of *P. tomentosa* [23], whereas the constituents responsible for the antibiosis effect were not identified.

4. Conclusion

The current research has demonstrated significant repellent and contact effects of *Plectranthus tomentosa* extract on *Tetranychus kanzawai*, with the level of control increasing with time, suggesting that intercropping *Plectranthus tomentosa* with tea bushes in commercial tea gardens may help control *Tetranychus kanzawai*, as may an acaricide-like extract from *Plectranthus tomentosa.* Such strategies may play a major role in the future in achieving pollution-free prevention and control of mites (possibly also mites other than *Tetranychus kanzawai*) in tea gardens, providing references for the large-scale planting of insecticidal plants and the biomimetic synthesis of pesticides.

Data Availability

The data used to support the results of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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