Bioactivity of Garlic-Straw Extracts Against the Spider Mites, *Tetranychus urticae* and *T. viennensis*[1]

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ABSTRACT The twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), and the hawthorn spider mite, *T. viennensis* Zacher, are two of the most important pest mites of apple trees (Rosaceae) in China. Because garlic straw, *Allium sativum* L. (Amaryllidaceae), is normally dumped on farmland, it potentially represents a cheap and readily available resource for pest management. The contact toxicity and repellent effects of garlic-straw extracts (20, 10, 5, 2.5, and 1.25 g/L) were tested against female adults of *T. urticae* and *T. viennensis* in the laboratory. The 20 g/L concentration caused 76.5% and 54.9% mortality 48 h after treatment on *T. urticae* and *T. viennensis*, respectively. The toxicity regression equations for garlic-straw extract on *T. urticae* and *T. viennensis* 48 h after treatment were y = 1.3 x + 3.9 and y = 0.8 x + 4.1, and the LC_{50} values were 7.2 g/L and 13.8 g/L, respectively. Repellency of *T. urticae* was 95.6% and 65.2% after 24 h at extract concentrations of 10 g/L and 20 g/L, respectively. The other concentrations of the garlic-straw extract had no significant repellent effects. However, each concentration of the garlic-straw extract had significant repellent effects on *T. viennensis*, and the repellent rates were higher than 90% for each concentration. Garlic straw or extracts from it may have potential uses for the sustainable management of *T. urticae* and *T. viennensis*.

KEY WORDS Tetranychidae, biological activity, ethanol extract

The twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most important world-wide pests of fruits, vegetables, and ornamental plants (Ho 2000, Raworth 2001, Takafuji et al. 2000). This mite damages around 1200 plant species, including apple trees (Rosaceae) in China (Zhang 2003). The hawthorn spider mite, *Tetranychus viennensis* Zacher, another widely distributed pest, also can cause severe damage to apple trees in China (Shi et al. 2004).

Control of *T. urticae* and *T. viennensis* with conventional acaricides is particularly difficult because of these mites' abilities to quickly develop resistance, and resistance to more than 80 acaracides from over 60 countries has been reported (Martison et al. 1991, Kim et al. 1999, Badawy et al. 2010). Abuse of synthetic acaricides can lead to serious adverse effects against humans and the environment, as well as non-target organisms, including beneficial

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insects and mites that prey on pests (Kumral et al. 2010). Because of this problem, researchers are now focusing on alternatives to chemical acaricides.

Certain plants have been suggested as alternative sources of chemicals for pest control because of their low mammalian toxicity compared with synthetic insecticides (Ahmed et al. 1984). Kim et al. (2005) evaluated the effects of crude plant extracts on *T. urticae, Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae). The repellent effect of garlic extract, *Allium sativum* L. (Amaryllidaceae), on *T. urticae* was previously reported by Boyd & Alverson (2000). Attia et al. (2011) showed that the extract of garlic led to a rise in female mortality and a reduction in fecundity of *T. urticae* with increasing concentration. Cheng et al. (2008) documented the inhibitory effects of garlic-straw aqueous extracts against three pathogens. However, there are no reports on the bioactivity of garlic straw or extracts from it against mites. In this study, we studied the contact toxicity and repellent effects of an ethanol extract of garlic straw on female adults of *T. urticae* and *T. viennensis.*

Materials and Methods

Mite colony. The original populations of *T. urticae* and *T. viennensis* were obtained from apple orchards at the Zhengzhou Fruit Research Institute, Zhengzhou, China. The colony of *T. urticae* was reared on peanut leaves, *Arachis hypogaea* L. (Fabaceae), under pesticide-free laboratory conditions at 26 ± 1.8°C, 75 ± 5% relative humidity, and 14:10 h (light:dark) photoperiod. Ten mated female adult *T. urticae* were placed on fresh un-infested peanut leaves and removed after 24 h with a fine brush. Recently emerged female adults that hatched from eggs on these leaves were used in all experiments. The colony of *T. viennensis* was reared on leaves of Japanese cherry, *Prunus serrulata* Lindl. (Rosaceae), under laboratory conditions identical to those used for *T. urticae.*

Extracts of garlic straw. Garlic straw was collected from an experimental field at the Zhengzhou Fruit Research Institute in April 2013. The straw was dried in an oven at 40°C and powdered with a grinder. Plant materials (50 g each) were extracted twice with 500 mL of 95% ethanol for two days in the laboratory. After filtering through a Buchner funnel, the filtrates were concentrated to dryness with a rotary evaporator at 40°C.

Assay for contact toxicity of ethanol extracts. The extracts were diluted to 100 g/L in absolute acetone, and then the test solutions were adjusted to the required concentrations using distilled water. The control solution was 10% acetone in distilled water. The slide-dip method recommended by the Food and Agriculture Organization (FAO) was used (Busvine 1980). Female adults were placed dorsally on pieces (1 cm^2) of double-coated tape stuck to glass microscope slides, with 30 mites on each slide. These sides were dipped into the test or control solution for 5 s, and then air dried. The slides were kept at 26 ± 1.8°C, 75 ± 5% relative humidity, and 14:10 h light:dark in an incubator. The total numbers of live and dead adult mites were checked under a binocular microscope 24 h, 48 h, and 72 h after treatment. The adult mites were considered dead if no movement was apparent after probing with the tip of a fine brush. Corrected mortality of female adults was calculated by Abbott's formula (Abbott 1925). All treatments were replicated three times.

Repellency test of ethanol extracts against *T. urticae*. The methods we used for the repellency tests of the ethanol extracts of garlic straw against *T. urticae* were similar to those described by Roh et al. (2012). One of two peanut leaves was dipped into the test solution for 10 s, and the other leaf was used as an untreated control. After the two leaves were air-dried, 20 female *T. urticae* adults were placed onto the petiole between the two leaves. The leaves were arranged randomly in an air-conditioned laboratory (26 ± 1.8°C, 75 ± 5% relative humidity, and 14:10 h light:dark) to remove the influence of visual cues such as light on the position of the twospotted spider mites. The numbers of adult mites on the treated and un-treated leaves were counted after 24 h. The numbers of eggs on both sides of the leaf surfaces also were counted under a dissecting microscope. This experiment was replicated five times. The repellency percentage (% R) of the extracts was calculated as:

$$\% \mathrm{R} = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C}} \times 100,$$

where T is the number of mites in the treatment, and C is the number of mites in the control.

Repellency test of ethanol extracts against *T. viennensis*. The repellency test of ethanol extracts of garlic straw against *T. viennensis* was similar to the method for *T. urticae,* above. Japanese cherry leaves that were cut into symmetrical shapes were used to evaluate the repellency of the extracts. Half of each leaf was immersed into a test solution for 10 s and the other half of the leaf served as an untreated control. After air-drying, 20 female *T. viennensis* adults were introduced onto the petiole of the leaf. The leaves were arranged randomly in an air-conditioned laboratory (26 ± 1.8°C, 75 ± 5% relative humidity, and 14:10 h light:dark) and rotated to remove the influence of visual cues such as light on the position of the mites. Using a dissecting microscope, the numbers of mites and eggs on each half of treated and un-treated leaves were counted 24 h after treatment. All treatments were repeated five times. The repellency percentage (% R) of the extracts was calculated according to the equation shown above.

Statistical analysis. Mortality and repellency-rate data were transformed to arcsine square root before analysis of variance (ANOVA), and significant differences among means were compared by Duncan's new multiple ranges test using SAS 9.1 software (SAS Institute, Cary, NC). The LC_{50} values for mortality were calculated using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA).

Results

Contact toxicity of ethanol extracts. The activities of ethanol extracts of garlic straw (20, 10, 5, 2.5 and 1.25 g/L) against female adults of *T. urticae* and *T. viennensis* are shown in Tables 1 and 2. After 48 h, the highest concentration of garlic-straw extract (20 g/L) caused the highest mortality to adult female *T. urticae* (76.5%) and *T. viennensis* (77.8%).

The regression equations for toxicity of the garlic-straw extract on adult females were calculated 48 h after the mites were placed on the leaves. These equations were $y = 3.9 + 1.3x$ and $y = 4.1 + 0.8x$ for *T. urticae* and *T. viennensis*, respectively (Table 3). Mortality increased in a time-dependent and concentration-dependent

Table 1. Contact toxicity of ethanol extracts of garlic straw against female *T. urticae* adults.

	Mortality (%) (Mean ± SE)			Corrected mortality (%) (Mean ± SE)		
Concentration (g/L)	24 h	48 h	72 h	24 h	48 h	72 h
20.0	65.6 ± 2.2 a[1]	77.8 ± 7.8 a	95.6 ± 4.4 a	64.0 ± 1.4 a	76.5 ± 7.9 a	94.7 ± 6.06 a
10.0	26.7 ± 3.3 b	53.3 ± 11.7 b	100.0 ± 0.0 a	23.3 ± 1.51 b	50.6 ± 11.7 ab	100.0 ± 0.0 a
5.0	11.1 ± 2.9 c	41.1 ± 13.1 bc	95.6 ± 4.4 a	7.0 ± 5.9 c	37.7 ± 12.7 bc	94.7 ± 4.76 a
2.5	12.2 ± 4.8 c	36.7 ± 1.9 bc	84.4 ± 12.2 ab	8.1 ± 2.5 c	32.9 ± 1.0 bc	81.3 ± 14.65 ab
1.25	6.7 ± 1.9 c	21.1 ± 4.4 c	61.1 ± 1.1 b	2.3 ± 4.1 c	16.5 ± 6.0 c	53.3 ± 4.42 b
Untreated control	4.4 ± 2.9 c	5.6 ± 2.2 d	16.7 ± 5.8 c	—	—	—

[1]Different letters in the same column indicate a significant difference ($P = 0.05$) according to Duncan's multiple range test.

Table 2. Contact toxicity of ethanol extracts of garlic straw against female *T. viennensis* adults.

	Mortality (%) (Mean ± SE)			Corrected mortality (%) (Mean ± SE)		
Concentration (g/L)	24 h	48 h	72 h	24 h	48 h	72 h
20.0	30.3 ± 8.1 a[1]	59.6 ± 15.3 a	95.5 ± 3.1 a	27.9 ± 6.4 a	54.9 ± 14.8 a	89.4 ± 8.6 a
10.0	11.2 ± 4.1 b	48.3 ± 3.5 ab	93.3 ± 6.9 a	8.1 ± 2.9 b	42.4 ± 1.1 ab	84.2 ± 18.8 a
5.0	6.7 ± 4.0 b	47.2 ± 11.4 ab	95.5 ± 2.3 a	3.4 ± 2.0 b	41.1 ± 9.3 ab	89.4 ± 6.1 a
2.5	6.7 ± 5.1 b	34.8 ± 4.6 ab	100.0 ± 0.0 a	3.4 ± 7.1 b	27.3 ± 8.2 ab	100.0 ± 0.0 a
1.25	5.6 ± 4.0 b	26.7 ± 8.4 bc	94.4 ± 2.9 a	2.2 ± 1.2 b	18.2 ± 8.3 b	86.9 ± 7.8 a
Untreated control	3.5 ± 3 .3 b	10.3 ± 6.6 c	57.5 ± 5.4 b	—	—	—

[1]Different letters in the same column indicate a significant difference ($P = 0.05$) according to Duncan's multiple range test.

Table 3. LC_{50} values and regression equations for garlic-straw extracts against *T. urticae* and *T. viennensis* adult females after 48 h.

Mite species	Regression equation	Correlation coefficient	LC_{50} (95% confidence interval) (g/L)
T. urticae	y = 3.9 + 1.3 x	0.97	7.2 (5.6–9.1)
T. viennensis	y = 4.1 + 0.8 x	0.98	13.8 (8.4–22.6)

manner. The LC_{50} values were 7.2 and 13.8 g/L for *T. urticae* and *T. viennensis*, respectively (Table 3).

Repellent effects of ethanol extract. The garlic-straw ethanol extracts significantly repelled female *T. urticae* adults at the two highest concentrations (65.2% for 20 g/L, and 95.6% for 10 g/L) (Table 4). At these two concentrations, there was a significant difference between the total number of *T. urticae* eggs on treated and un-treated leaves. All concentrations of the garlic-straw extracts repelled female *T. viennensis* at rates higher than 90% (Table 5).

Discussion

In this study, we assessed the contact toxicity and repellent effects of the garlic-straw ethanol extract against female adults of *Tetranychus urticae* and *T. viennensis*. The bioactivities of ethanol extracts from different plants against these mites have been reported by several researchers. For example, formulated neem seed-kernel extracts, *Azadirachta indica* A. Juss (Meliaceae), were reported to influence mortality, repellency, and fecundity of mites (Monsuer & Ascher 1983, Monsuer et al. 1993, Dimetry et al. 1993). In addition, it was found that two commercial preparations of neem-seed extracts were effective on *T. urticae* (Mironova & Khorkhordin 1996, Dimetry et al. 1993). Antonious & Snyder (2006) reported that the extracts of wild tomato leaves (Solanaceae) showed strong repellent effects on *T. urticae*. Wang et al. (2007) stated that crude extract of walnut leaves, *Juglans regia* L. (Juglandaceae), had some contact and systemic effects on *T. cinnabarinus* (Boisduval) and *T. viennensis*. Extracts of yew (*Taxus* spp.) (Taxaceae) caused high mortality, a decrease in female fecundity, and shortened longevity of *T. urticae* (Furmanowa et al. 2001, 2002). Crude foliar extracts of 67 plant species from six subfamilies of Australian Lamiaceae showed both contact and systemic toxicity to these mites (Rasikari et al. 2005). Shi et al. (2004) reported the efficacy of extracts of *Stellera chamaejasme* L. (Thymelaceae) against *T. viennensis*. Shi et al. (2006) also reported the efficacy of herbaceous plant, *Kochia scoparia* (L.) Schrad (Macrophomina) (Amaranthaceae), on *T. urticae*, *T. cinnabarinus*, and *T. viennensis*, and stated that this extract had both contact and systemic toxicity on these mites.

The repellent effect of garlic extracts on *T. urticae* was previously reported by Boyd & Alverson (2000). Dabrowsky & Seredynska (2007) reported that garlic extract tested on *T. urticae* resulted in mortality of 48–57%. Attia et al. (2011) revealed that the extract of garlic led to a rise in female mortality and a reduction in fecundity with the increasing of concentration. Attia et al. (2012) also showed

Table 4. Repellent effects of ethanol extracts of garlic straw against *T. urticae*.

Concentration (g/L)	No. of treated mites	% of adults moved to leaves			Repellency rate (%)	Total No. of eggs		
		Treated	Untreated	*P* value		Treated	Untreated	*P* value
20.0	93	25.8	74.2*[1]	0.009	65.2 b[2]	148	1080*	0.033
10.0	94	4.3	95.7*	<0.0001	95.6 a	62	829*	0.033
5.0	86	32.6	67.4	0.149	51.8 b	274	858	0.096
2.5	87	42.5	57.5	0.468	26.0 c	310	815	0.185
1.25	90	48.9	51.1	0.908	4.4 d	413	618	0.408

[1]*Significant difference between treated and untreated by *t*-test ($P \leq 0.05$).
[2]Different letters in the same column indicate a significant difference ($P = 0.05$) level according to Duncan's multiple range test.

Table 5. Repellent effects of ethanol extracts of garlic straw against *T. viennensis*.

Concentration (g/L)	No. of treated mites	% of adults moved to leaves			Repellency rate (%)	Total No. of eggs		
		Treated	Untreated	*P* value		Treated	Untreated	*P* value
20.0	83	8.4	91.6*[1]	<0.0001	90.8 a[2]	24	495*	<0.0001
10.0	96	5.2	94.8*	<0.0001	94.5 a	25	537*	<0.0001
5.0	90	7.8	92.2*	0.0005	91.6 a	16	557*	<0.0001
2.5	99	7.1	92.9*	<0.0001	92.4 a	50	495*	<0.0001
1.25	92	3.3	96.7*	<0.0001	96.6 a	11	460*	0.0005

[1]*Significant difference between treated and untreated by *t*-test ($P \leq 0.05$).
[2]Different letters in the same column indicate a significant difference ($P = 0.05$) level according to Duncan's multiple range test.

that garlic-bulb extracts have acaricidal activity against *T. urticae*. Nour El-Deen et al. (2013) considered garlic-seed extract the most suitable materials for IPM because it caused high mortality for *T. urticae* with minimal effect on its predator mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). Cheng et al. (2008) showed the inhibitory effects of garlic-straw aqueous extracts against three plant pathogens. However, until this study, there have not been any reports on the efficacy of ethanol extracts of garlic straw against mites. We showed that ethanol extracts of garlic straw have bioactivity against *T. urticae* and *T. viennensis* under laboratory conditions. However, the isolation and identification of the effective compounds from garlic straw extracts and their practical use as an acaricide on the two mite species still needs to be researched. Because garlic straw is normally just dumped onto farmland, it represents a readily-available and less-costly resource compared with garlic bulbs, whose extracts have shown inhibitory effects on plant pathogens. If garlic-straw extract were used in Integrated Pest Management of apple orchards, then pollution caused by synthetic acaricides could be reduced with better ecological and economic benefits. Hence, garlic-straw extract may have potential as an inexpensive tool in the sustainable management of *T. urticae* and *T. viennensis* in apple orchards.

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