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Acaricidal and repellent effects of *Aloe vera* L. leaf extracts against *Tetranychus urticae* Koch (Acari: (Tetranychidae)

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Abstract
The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a cosmopolitan pest that causes considerable damage to vegetables, fruit and flower plants. The acaricidal, ovicidal and repellent activity of four different extracts of *Aloe vera* L. (Asphodelaceae) leaves were evaluated against *T. urticae* under laboratory conditions. Four different solvents of *A. vera* leaf extracts were tested against *T. urticae* at concentrations of 0.25, 0.50, 1.0 and 2.0%. The results indicated that all extracts had lethal and repellent effects on *T. urticae*. The acetone extract showed the highest mortality (95.0%) of adult females followed by ethanol (83.0%) at 2.0% concentration. The LC_{50} values of acetone, ethanol, methanol and petroleum ether extract for adult females were 0.446, 0.667, 0.953 and 1.279, respectively and for eggs were 0.950, 1.406, 2.115 and 3.312, respectively. The ethanol extract was found to be more effective as a repellent against adult females of *T. urticae* followed by acetone, methanol and petroleum ether causing a reduction in egg production per female by 96.0, 94.0, 85.0 and 83.0%, respectively. In the residual test, the acetone extract showed the highest mortality (49%) after 1 hour of treatment followed by ethanol (43.0%), methanol (29.0%) and petroleum ether (25.0%). The percent of mortality decreased after 48 and 72 hours after treatments. The results show that *A. vera* has a high potential to become a botanical acaricide for controlling *T. urticae*.

Keywords: *A. vera*, *T. urticae*, acaricidal activity, ovicidal activity, repellency

Introduction
The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae) is known as a phytophagous pest of many cultivated crops throughout the world. This herbivore attacked a variety of cultivated crops and feeds over 1100 plant species in 140 families of economic importance value [1]. Both immature and adults of *T. urticae* suck sap from the lower surface of leaves [2]. Leaf curling, yellowing, bronzing, defoliation and even plant death occur due to the direct effect of mites feeding. Indirect effects of feeding result in reduced photosynthesis, transpiration, loss of green arena and ultimately, significant yield loss [3, 4]. In the last few decades, control of the population of *T. urticae* has relied heavily on the continued use of acaricides or pesticides. A major problem with this pest is its ability to quickly develop resistance to certain acaricides or pesticides [5]. Besides resistance, the widespread use of traditional pesticides against mites can have serious effects on beneficial organisms, human health and the environment [6]. In order to achieve sustainable management of TSSM, it is important to reduce the use of synthetic acaricide or pesticides having a different mode of action [7, 8]. Many plant extracts have a wide biological activity against insects and mites together with toxicity, repellence, oviposition deterrence, feeding and growth inhibition activity [9]. Unlike pesticides, numerous plant extracts have higher efficacy on pests, fast biodegradability, cheaper and have less impact on the environment [10, 11]. Extensive research has been focused on the insecticidal activity of plant metabolites, which affect survival, growth, germination, nutrition, and various anatomical and biological processes of insects [12, 13].
Aloe vera L., is a perennial herbaceous plant in the family Asphodelaceae. Known as medicinal aloe, its medicinal properties are widely recognized by traditional herbal medicine doctors and practitioners. *Aloe vera* plant contains over 160 chemical components and is used in 80 or more medicinal ingredients [14, 15].

Aloin, one of the compounds, has antimicrobial, antifungal antibiotics, anti-oxidant activities and also plays an important role in the destruction of pests in granaries [16] however, only toxicity and ovicidal activity have been recognized [17]. With respect to the benefits of aloe for humans, little is known about insecticidal and/or acaricidal activities. Therefore, the purpose of this study was to assess the acaricidal and repellent activity of *A. vera* leaf extracts against *T. urticae.*

Materials and Methods

The study was conducted from October 2017 to June 2018 in the laboratory of the Department of Entomology, Faculty of Agriculture, Hajee Mohammad Danesh Science and Technology University (HSTU) in Dinajpur, Bangladesh.

Mite collection and rearing

The adult mites, *Tetranychus urticae* were collected from an infested bean field of Hajee Mohammad Danesh Science & Technology University campus, in 2017. Fifty percent of the collected mites were introduced on the country bean leaves grown in several plastic pots (20 D × 20 H cm) and placed in the front of the laboratory. Another fifty percent of the mites were reared on bean leaves in Petri dishes (9 D × 2 H cm). Fresh bean leaves were provided to replace the old dried leaves in the Petri dishes.

Collection of plant materials

Mature fresh *Aloe vera* L. leaves were collected from Anando nursery, Paharpur, Dinajpur, Bangladesh.

Chemical Reagent

Analytical grades of methanol, ethanol, acetone, petroleum ether were collected from (Daejung chemicals and metals Co. Ltd., Korea), (Merck KGaA, Germany), (Sigma-Aldrich Co.).

Preparation of plant extracts

Aloe vera leaves were collected and dried in the shade. After that, the leaves were cut into small pieces and finally dried again in an oven at a temperature of 50 ˚C. The dried plant materials were ground to make fine powders of 80 meshes for extraction with four different solvents (acetone, ethanol, methanol and petroleum ether). 100 grams of *A. vera* powder was taken in a 600 ml beaker and 300 ml of acetone, ethanol, methanol and petroleum ether were added respectively. The mixtures were stirred by a magnetic stirrer (600 rpm) for 30 minutes and shaken by hand and allowed to stand for 72 hours. After that, the mixtures were filtered through filter paper (Whatman No. 1). The filtered materials were taken into a volumetric flask and the solvents were allowed to evaporate with the aid of a rotary evaporator (50-60 ºC). Finally, the crude extracts were stored in tightly corked glass vials and stored in the refrigerator at 3 ºC for experimental use.

Acaricidal effect on adults

A group of 20 *T. urticae* (24 h old) females was randomly selected from the stock culture and transferred to fresh bean leaf discs (3 cm in diameter) placed adaxial side up on moistened cotton in Petri dishes (9 D × 2 H). Leaf discs were sprayed with four different solvent extracts of *A. vera* at four different concentrations (0.25, 0.50, 1.0, and 2.0%). A control test was considered performed using distilled water. Each treatment was repeated 4 times. A stereomicroscope (BST 606, Made in Germany) was used in this study to observe the alive and dead mites. The mites were considered dead if their appendages did not move when probed with a fine brush 24 h post-treatment. The mortality rate was calculated by using Abbott's corrected formula [18].

Ovicidal effect

A bean leaf disc of approximately 3 cm in diameter was used as a substrate to oviposit. In each treatment, four leaf discs were used and fifteen female mites were placed upside down on hydrophilic cotton soaked in water in a Petri dish (9 D × 2 H) and allowed to 5 hours to lay eggs. After 5 hours the adults were removed and the eggs were checked to ensure that at least 25 eggs, less than 24 h old will be laid on each disc. The leaf with egg were treated with four different concentrations (0.25, 0.50, 1.0and 2.0%) of four different solvent extracts of *A. vera* with the help of a hand sprayer. A water spray control was also maintained. The eggs were observed for seven days till hatched. The numbers of hatched and non hatched eggs were recorded using a stereomicroscope.

Repellency effect

Leaf disc of bean along with midrib of 3 cm in diameter was used to evaluate the repellency of the four different solvent extracts of *A. vera* mixture. One part of the disc was dipped in the test concentrations of 10 seconds and the other half was used as a control. The treated discs were allowed to air dry for 30 minutes and then placed on moistened cotton in Petri dishes. Twenty adult females were put on the midrib of each disc. The experiment was replicated four times. The number of adult mites present on each half of the disc was recorded 24 hours after mite transfer. The number of eggs laid on both sides was also recorded under a stereomicroscope. The data were expressed as percentage repellency (PR) which was computed using the following formula described by McDonald *et al.* [19] with some modifications.

$PR\ (\%) = (N_C - 50) \times 2$

Where, N_C is the percentage of mites present in the untreated portion of the disc. Positive (+) values expressed repellency and negative (-) values attractancy. The mean values were then categorized according to different class using the following range. Percent repellency > 0.01 to < 0.1 = class 0; 0.1to 20 = class I; 20.1to 40 = class II; 40.1 to 60 = class III; 60.1to 80=class IV; 80.1to100 = class V [20].

Residual or persistence test

The LC_{50} dose of four different solvent extracts of *A. vera* leaves was applied on the bean leaf disc (3 cm diameter) with a hand sprayer. After 1, 24, 48 and 72 h of treatment 20 adult females of *T. urticae* were carefully released on the treated leaf discs. Each treatment was repeated 4 times. These mites were considered dead when they were unable to move even after probing with a brush.

Statistical analyses

Differences in mortality among treatments were analyzed for one way ANOVA using SPSS program Version 20.0 and means were compared using Tukey's test at $P<0.05$. Probit analysis was used to determine LD_{50} and LD_{90} using Polo Plus software [21].

Results and Discussion

Adult mortality of *T. urticae* of four different solvent extracts

Acaricidal activity of four different solvent extracts of *A. vera* leaves was tested against *T. urticae* at four different concentrations (0.25, 0.50, 1.0 and 2.0%). Data presented in

Table 1 indicates that, acetone extract of *A. vera* showed strong acaricidal activity against *T. urticae* followed by ethanol, methanol and petroleum ether extracts. At 0.25% concentration, acetone and ethanol extracts showed significantly higher acaricidal activity over control but there were no significant differences observed among the extracts. At 0.50% concentration acetone solvent extract exhibits higher mortality of mites which is significantly different from other extracts and control except for ethanol extract. At 1.0% concentration mortalities in the four solvent extract treatments increased considerably compared to those at 0.25 and 0.5% concentration and acetone solvent extract were showed the highest mortality. At 2.0% concentration acetone extract achieved 94.81% mortality, followed by ethanol (81.82%), methanol (71.43%) and petroleum ether (61.04%) (Table 1). The acetone solvent extract of *A. vera* was found to have good contact acaricidal activity against the *T. urticae* of female adult mites. Wei *et al.* [22] stated that 24 h after treatment, the acetone extract of *A. vera* leaves showed the strongest acaricidal activity against *T. cinnabarinus* female adults. These findings were also supported by Zhang *et al.* [23], who reported that the acetone extract of *A. vera* has a strong acaricidal activity against *T. cinnabarinus* female. Zhang *et al.* [24] opined that acetone extract of *A. vera* leaves has acaricidal activity against *T. cinnabarinus* and *P. citri*. Amer *et al.* [25] revealed that ethyl alcohol and acetone extracts were the most potent extracts against adult females of *T. urticae*.

Table 1: Adult mortality of *A. vera* leaf extracts at different concentrations recorded 24 h after exposure (Mean ± SE) (%)

Extracts	% Concentrations			
	0.25	**0.50**	**1.0**	**2.0**
Acetone	30.00 ± 4.56a (27.27)	48.75 ± 5.54a (46.75)	82.50 ± 3.22a (81.82)	95.00 ± 3.53a (94.81)
Ethanol	22.50 ± 3.22a (19.48)	35.00 ± 4.56ab (32.47)	66.25 ± 4.26b (64.94)	82.50 ± .22ab (81.82)
Methanol	18.75 ± .26ab (15.58)	28.75 ± 4.26b (25.97)	50.00 ± 4.56c (48.05)	72.50 ± 3.22bc (71.43)
Petroleum ether	17.50 ± 3.22ab (14.29)	23.75 ± 4.26b (20.78)	43.75 ± 2.39c (41.56)	62.50 ± 5.20c (61.04)
Control	3.75 ± 2.39b	3.75 ± 2.39c	3.75 ± 2.39d	3.75 ± 2.39d

Values in the parenthesis are corrected mortality; values in the same column with a common letter are not significantly different at $P<0.05$ (Tukey's HSD test).

Ovicidal activity of four different solvent extracts of *A. vera* against *T. urticae*

Ovicidal activities of four different solvent extracts were presented in Table 2. At a concentration of 0.25%, the highest mortality was observed in acetone extract, which is significantly different from control. All extracts showed significant egg mortality over control, with the exception of petroleum ether extract at the concentration of 0.50%. Acetone extract showed higher mortality of eggs which is significantly different from other extracts at 1.0% concentration and control but no significant difference was observed between acetone and ethanol aloe extracts. At a concentration of 2%, the acetone extract reached a mortality rate of 65.0% followed by ethanol (57.0%), methanol (49.0%) and the extract of petroleum ether (40.0%). The ovicidal activities of acetone extract of the *A. vera* leaves have a good effect against *T. urticae.* Zhang *et al*. [23] indicated that the acetone extracts of *A. vera* have higher oviposition activity against *T. cinnabarinus*. Kumral *et al.* [26] also reported that ethanolic extracts from leaves and seeds of *D. stramonium* have acaricidal, oviposition deterrent activities against *T. urticae*. The egg mortality of *T. urticae* using methanol extract of river red gum *Eucalyptus camaldulensis* leaves was 63.26% [27]. Hussen *et al*. [28] reported that ethanol extract from the fruit of *Capparis aegyptia* was the least effective extract against *T. urticae* eggs. Derbalah *et al*. [29] discovered that *Nigella sativa* Linn. (Ranunculaceae) (seeds) and *Artemisia cina* L. (leaf) (Asteraceae) extracts were toxic to eggs of *T. urticae* with LC_{50} values of 1850.92 and 2740.42 ppm. Ghaderi *et al*. [30] observed that the ovicidal activity of methanolic extracts of *S. meifolia*, *A. orientale*, *T. elliptica* and *P. viscosa* against *T. urticae* eggs were 45.84, 41.40, 40.11 and 37.66 % respectively. Auamcharoen *et al*. [31] stated that crude methanol extracts of *D. grandiflora* extracts showed moderate repellency and also inhibited egg production in this mite species.

Table 2: Ovicidal activity of *A. vera* leaf extracts at different concentrations recorded 7 days after exposer (Mean ± SE) (%)

Extracts	% Concentration			
	0.25	**0.50**	**1.0**	**2.0**
Acetone	18.00±4.16a	32.00±1.63a	58.00±5.29a	65.00±4.12a
Ethanol	14.00±4.16ab	22.00±5.29a	46.00±3.46ab	57.00±5.00ab
Methanol	11.00±4.43ab	19.00±6.19a	39.00±3.41bc	49.00±2.51bc
Petroleum ether	11.00±3.41ab	15.00±3.41ab	29.00±3.41c	40.00±3.65c
Control	0.00±0.00b	0.00±0.00b	0.00±0.00d	0.00±0.00d

Values in the same column with a common letter are not significantly different at $P<0.05$ (Tukey's HSD test)

Toxicity of four different solvent extracts of *A. vera* to *T. urticae* adults by topical spray

Acetone extracts of *A. vera* showed the highest acaricidal activities followed by ethanol, methanol and petroleum ether extracts (Table 3). The LC_{50} values after 24 h for adults were 0.45, 0.67, 0.95 and 1.28 respectively, while for eggs 0.95, 1.41, 2.12 and 3.31 were recorded after 7 days. The slope values of the regression line were 2.46, 1.97, 1.68 and 1.46 for adults and 1.51, 1.48, 1.35 and 1.14 for eggs respectively. LC_{90} values were 1.48, 3.00, 5.51 and 9.72 for adults and 6.68, 10.32, 18.75, 43.67 for eggs respectively (Table 3).

Zhang *et al*. [23] found that the LC_{50} value of aloe acetone extract on *T. cinnabarinus* female were 0.836 and 0.167 mg/ml for 42h and 72h, respectively. Wei *et al.* [22] also

reported the LC_{50} values of aloe acetone extract against *T. cinnabarinus* were 0.614 and 0.099 mg/ml for 42h and 72h, respectively. Wang *et al.* [32], tested *Juglans regia* L. leaf extracts with different solvents and reported the LC_{50} values of 730, 1660, 4960, 7450 and 9910 ppm for the extract of petroleum ether, chloroform, ethyl acetate, methanol, and distilled water, respectively.

Table 3: Statistical comparison of LC_{50} values of four different *A. vera* extracts against *T. urticae* adults and eggs

	Acetone		Ethanol		Methanol		Petroleum Ether	
	Adult	Egg	Adult	Egg	Adult	Egg	Adult	Egg
LC_{50} (%)	0.446	0.950	0.667	1.406	0.953	2.115	1.279	3.312
Lower limit	0.375	0.558	0.556	1.122	0.780	1.560	1.002	2.118
Upper limit	0.518	2.328	0.796	1.936	1.209	3.505	1.815	8.100
Slope ± SE	2.459 ± 0.27	1.514 ± 0.20	1.966 ± 0.24	1.480 ± 0.21	1.681 ± 0.23	1.352 ± 0.22	1.455 ± 0.23	1.144 ± 0.22
χ^2 (df)	1.838(2)	2.623(2)	1.470(2)	1.676(2)	0.881(2)	0.077(2)	1.025(2)	0.614 (2)
LC_{90} (%)	1.481	6.676	2.994	10.324	5.518	18.746	9.722	43.667

The original insect mortality data were corrected by Abbott's (1925) formula before analysis, χ^2 = Goodness of fit, df= Degrees of freedom, The tabulated value of χ^2 is 5.99 (df = 2)

Repellent effect of four different solvent extracts of *A. vera* against *T. urticae*

The effects of the four different extracts of *A. vera* leaves and doses are presented in Table 4. Of the extracts, ethanol showed the highest repellency effect (95.00%) followed by acetone (87.50%), methanol (77.50%), petroleum ether (70.00%) at a 2% concentration. The number of eggs laid by females significantly decrease compared to the control. For egg laying the repellency effects of acetone, methanol and petroleum ether extracts were statistically at par compared to the ethanol. Kumral *et al.* [26] reported that ethanol extracts from *D. stramonium* leaf and seed have repellent and reproduction inhibition activities against *T. urticae*. Kundu *et al.* [33] investigated the repellent effect of biskatali plant extract in two solvents namely ethyl alcohol and chloroform against red flour beetle. They found that ethyl alcohol and chloroform have a repellent effect on *Tribolium castaneum*. Antonious *et al.* [34] found that ethanol extracts of wild tomato leaves have been found to have a strong repellency effect on *T. urticae*. Hussein *et al.* [28] observed that a higher percentage of repellency was recorded in the ethanol extract of fruits and leaves (96.42 and 86.67 %) of *Capparis aegyptia*. El-Sharabasy [35] evaluated the repellent effect of crude extracts of *A. judaica* L. against adult females and immature stage of *T. urticae*. They found that leaf extraction with ethanol was most effective as a repellent effect against adult females and immature stage of *T. urticae* followed by acetone, petroleum ether and aqueous extraction. Teklay *et al.* [36] studied the insecticidal and repellent properties of garlic, tobacco, neem and *Aloe vera* extracts. All the plant extracts repelled the insects completely. Mostafa *et al.* [37] reported that the acetone, methanol and water extracts from mahagoni (*Swietenia mahagoni)* leaf showed larvicidal, antifeedant and insect repellent activity against the red flour beetle, *T. castaneum.*

Table 4: Repellent effect of four different *A. vera* leaf extracts against of *T. urticae* after 24 h of exposure

Solvent	% Concentrations	% Repellency	Repellency Class	Average no. of eggs/ female after 24 h	
				Treated	Control
Acetone	0.25	65	IV	2.75bc	43.13
	0.50	75	IV		
	1.0	85	V		
	2.0	87.5	V		
Ethanol	0.25	77.5	IV	1.87c	45.12
	0.50	80	IV		
	1.0	82.5	V		
	2.0	95	V		
Methanol	0.25	45	III	5.18bc	34.43
	0.50	55	III		
	1.0	70	IV		
	2.0	77.5	IV		
Petroleum ether	0.25	45	III	7.06b	41.18
	0.50	52.5	III		
	1.0	62.5	IV		
	2.0	70	IV		
Water		35	II	36.25a	51.00

Means in the same column followed by the different letters are significantly different at $P<0.05$ (Tukey's test)

Residual effects of four different solvent extracts of *A. vera* against *T. urticae*

The residual effects of the acetone**,** ethanol, methanol and petroleum ether extracts were presented in Figure 1. In the residual test, the mortality of *T. urticae* varied appreciably. One hour after treatment, acetone extracts show higher mortality (48.75%) followed by ethanol (42.50%), methanol (28.75%) and petroleum ether extract (25.00%). Twenty four hours after treatment, all extracts showed significantly different mortality rates than the untreated control. Acetone has a higher mortality rate than all other treatments. However, there was no significant difference was observed among acetone and ethanol, methanol and petroleum ether extract, respectively. The mortality percentage decreased after 48 and 72 hours of treatments.

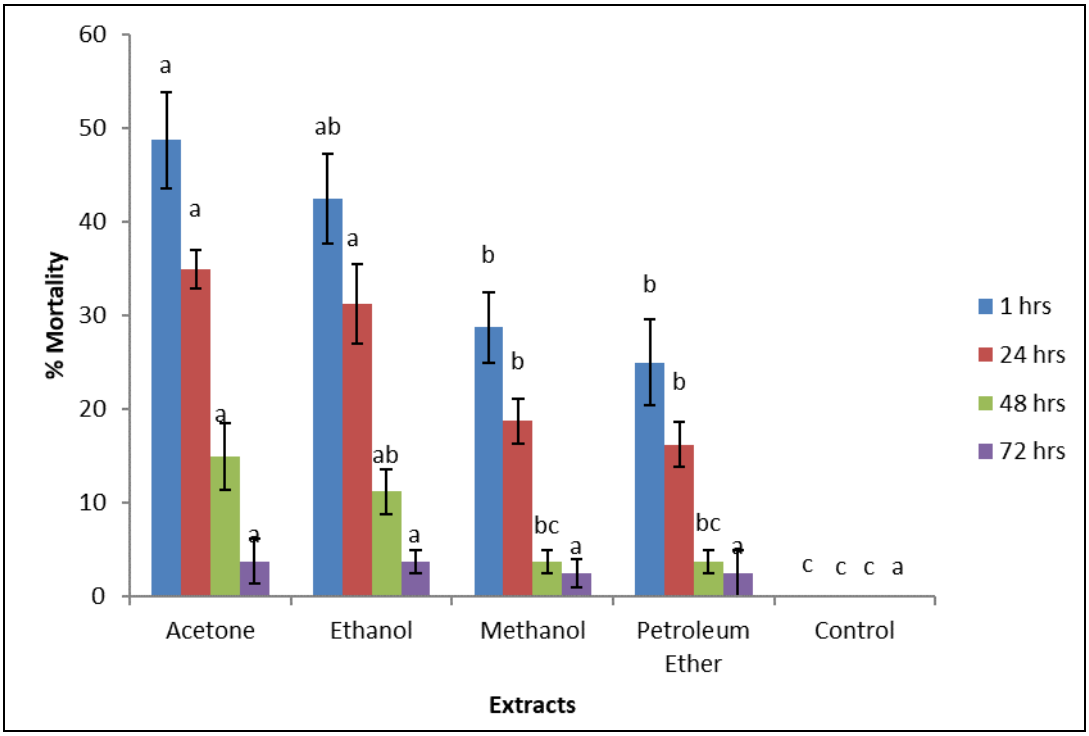

Fig 1: Persistence of *A. vera* leaf extracts against *T. urticae* at 1,24,48 and 72 hours old residue of LC_{50} values. [Bars marked with same letter do not differ significantly (P<0.05)]

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