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Research Paper

Wood vinegar from broadleaf tree bark carbonized at low temperature has exterminating effect on red mites by invading into their bodies

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ABSTRACT

In vitro experiments and histological studies were performed to investigate whether wood vinegar (WV) has an insecticidal effect on red mites. In the in vitro trials, red mites were entered into the test tubes including 18 ml of water or WV containing 18 to 1,800 µl to estimate the median WV 50% lethal concentration (LC50) for red mites. The LC50 was estimated to be 18.9 µl/cm3. On adding red mites to a Petri dish volatilized with acetic acid or WV, it was observed that the extermination effect of another component of WV on red mites was stronger than that of acetic acid. The analysis of WV components revealed high mineral content and a higher osmotic pressure (650 mOsm/L) than that of water (285 mOsm/L). After adult red mites were immersed in WV, they swelled, and black mineral particles of WV were found in their bodies. Body fluid exuding from young protonymph bodies was observed when the WV mineral powder was sprinkled on them. The inside contents were also exuding from eggs of red mites by spraying WV.. These results suggest that several types of WV mineral particles entered the red mite body by passing through the stigma and peritreme by respiration, and that the high osmotic pressure of WV entering into red mites may lead to death.

Key words: Broadleaf tree, microscopy, red mite, wood vinegar.

INTRODUCTION

In order to efficiently produce high-quality poultry products, there have been several types of nutritional studies focussing on the development of new feed ingredients. Further, to obtain safe and reliable poultry products, chickens must be bred under good environmental condition. However, in the current egg layer breeding method, hens were maintained at high population densities in a large-scale cage feeding system, thereby, increasing the infestation by red mites, *Dermanyssus gallinae* (De Geer, 1778), which are regarded as the most important and economically deleterious ectoparasites of layer hens worldwide. The red mite induces a reduction in egg production and quality by staining the egg surface with bloody feaces (Cencek, 2003) leading to poor sales (Chauve, 1998). Large numbers of mites can cause enormous blood loss, viral disease (Abdel-Ghaffaret al., 2008a, b; Mehlhorn, 2008; Valiente-Moro et al., 2007) and can induce severe anaemia and associated mortality (Kirkwood, 1967). These consequences of infestation by red mites are important for the welfare of chickens and economic sustainability.

The most common method for eliminating red mites from infested poultry farms is the application of pesticides. Even in organic farms, synthetic acaricides are used as the last resort for eliminating red mites. Insecticides, such as carbaryl, diazinon and permethrin, or fumigants, such as sulphur dioxide (Kim et al., 2007) have principally been used for controlling red mites. However, the use of such chemicals leads to the development of chemical resistance in red mites, chemical and antibiotic residues in the food and environmental contamination (Dalton and Mulcahy,

Figure 1. Dry distillation of bark from broadleaf trees in a flat kiln. New barks (brown) are placed above the previously carbonized barks (black); smoke is absorbed by the smoke flue at the bottom.

2001). Besides, the introduction of such several kinds of agro-chemicals has induced multiple-drug-resistant bacteria in poultry body. Faeces including the multiple-drug-resistant bacteria harm humans through vegetable and farm products. In the US, organophosphates such as chlorpyrifos and diazinon and carbamates such as carbaryl were phased out by the US Environmental Protection Agency under the 1996 Food Quality and Protection Act (US EPA, 2002).

Therefore, the development of alternatives to synthetic chemicals for eliminating red mites is required. Plants have been suggested as an alternative source for mite control products because some are non-toxic and have few harmful effects on non-target organisms and the environment (Isman, 1995, 2001), particularly, fumigant repellent effects (Kim et al., 2007). Among the plant-derived products known for their acaricidal effects on red mites, wood vinegar (WV) is promising. Because it is a residue obtained from the production of wood charcoals; WV is likely to be inexpensive.

It has been previously mentioned that WV could exterminate red mites without reducing egg production (Yamauchi et al., 2014); however, the underlying mechanism remains unclear. In this study, we investigated the presumed reasons from the standpoint of the manufacturing methods and physical composition of WV. It is difficult to study red mites because they reside on the host only at night and hide in small cracks and crevices of poultry houses during the day. Therefore, here, we conducted an *in vitro* study on the effects of WV on red mites and their morphology.

MATERIALS AND METHODS

Preparation of WV by dry-distillation system

WV was produced by Miyazaki Midori Seiyaku Co., Ltd (Miyazaki, Japan) as follows. Bark from several broad leaf tree species such as oak, chinquapin, and *Machilus thunbergii* was separated from the woody part. The flat kiln to dry-distill bark was a 5 m × 5 m × 1 m concrete tank, and there were two lines of six flat kilns (Figure 1). Eight flat kilns were used, and the other kilns used to finish the

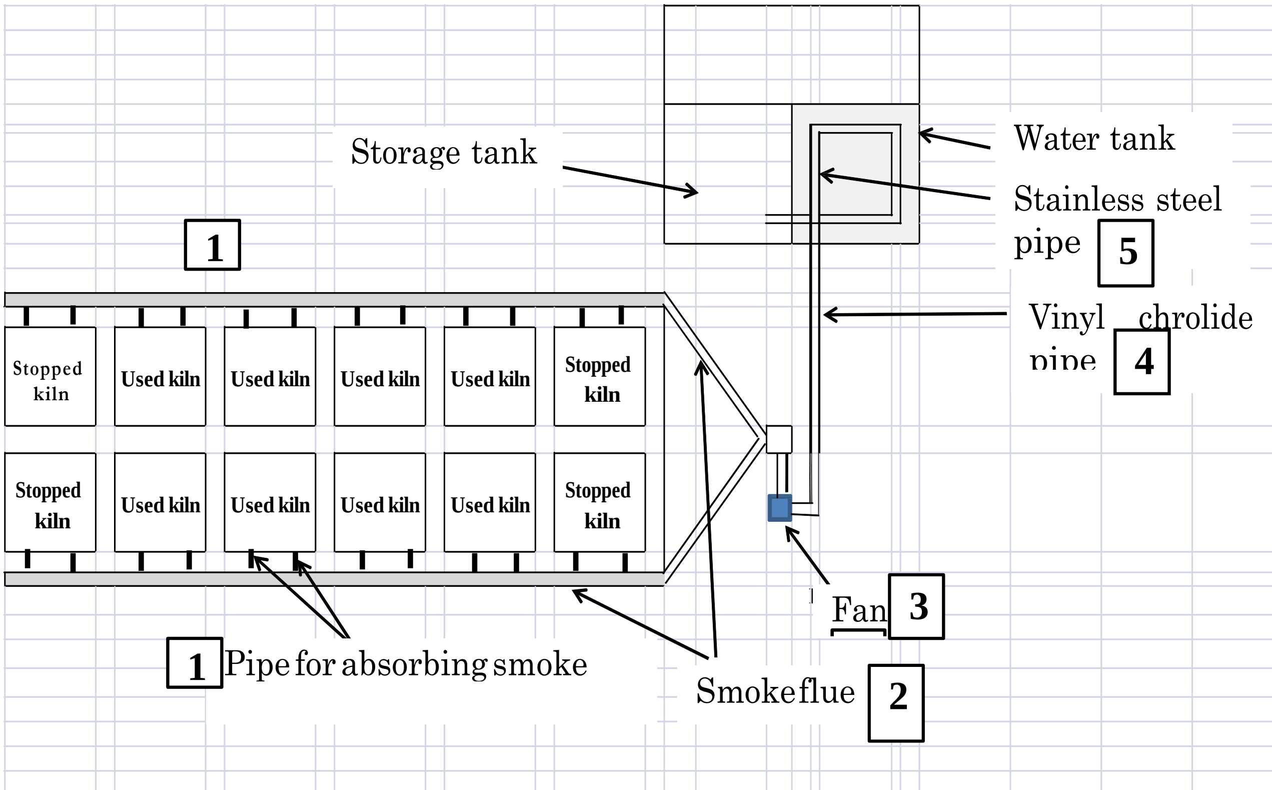

Figure 2. Ground plan of a flat kiln to dry-distill wood barks. The smoke moves along the route marked 1 to 5 in the figure.

charcoal and clean and prepare the next batch of charcoal. The bottom of each flat kiln had volcanic ash soil called Shirasu. Bricks were placed in the soil to support a pore-filled iron plate. The smoke-flue side wall of one of the concrete tanks had two 20 cm diameter holes to connect pipes for absorbing smoke at the bottom of the kiln between the soil and the iron plate (1 in Figure 2). The opposite side of the pipe was connected to the smoke flue (2 in Figure 2), which directed smoke to a fan (3 in Figure 2). After passing through the fan, the smoke was sent outside through a vinyl chloride pipe (4 in Figure 2) to a stainless pipe (5 in Figure 2) that circulated in the water tank. During circulation in the cool water tank, the smoke changed to an unpurified WV solution that remained in a storage tank for more than three months. The middle layer was collected as the WV, as several centimeter surface layer of unpurified WV solution included floating dust particles, and the bottom layer was tar.

A small amount of bark was placed into the flat kiln (including some bark embers as a pilot light) and ignited with a burner. After the fire was thoroughly spread to the first barks layer and it was carbonized, a second layer of bark was added until there was a 20 cm layer. The flat kiln was full in 3 weeks (Figure 1). Dry-distillation occurred using this dry-distillation system because of the low temperature of carbonization (300 to 400°C) and coordination of the oxygen quantity.

Experimental Design

1) Identifying the red mites by scanning electron microscopy (SEM)

Collected red mites were immersed in a chloroform solution for several seconds, and dried at room temperature for 5 min, and then immersed in a 5% ionic liquid (10 µl/mite, Hitachi HILEM IL 1000, Hitachi Ltd., Tokyo, Japan). The dried mites were fixed on an aluminum stub using double-sided carbon tape and observed by SEM (Hitachi TM 3030, Hitachi Ltd., Tokyo, Japan) without a platinum coating. According to the illustrations of Baker et al. (1956) and Nakamae (2001), the red mites were identified by their anus at the posterior part of the anal plate. In addition, the red mite SEM pictures were sent to a veterinary researcher for identification. Conventional morphology such as the stigma (respiratory pores) and peritreme were also observed (Wilson, 1964).

2) Estimation of the red mite WV 50% lethal concentration (LC_{50})

To estimate the approximate WV value of median lethal dose for red mites, 1,800 µl of water (bottle 1) and 18 (bottle 2), 180 (bottle 3), 450 (bottle 4), 900 (bottle 5), or

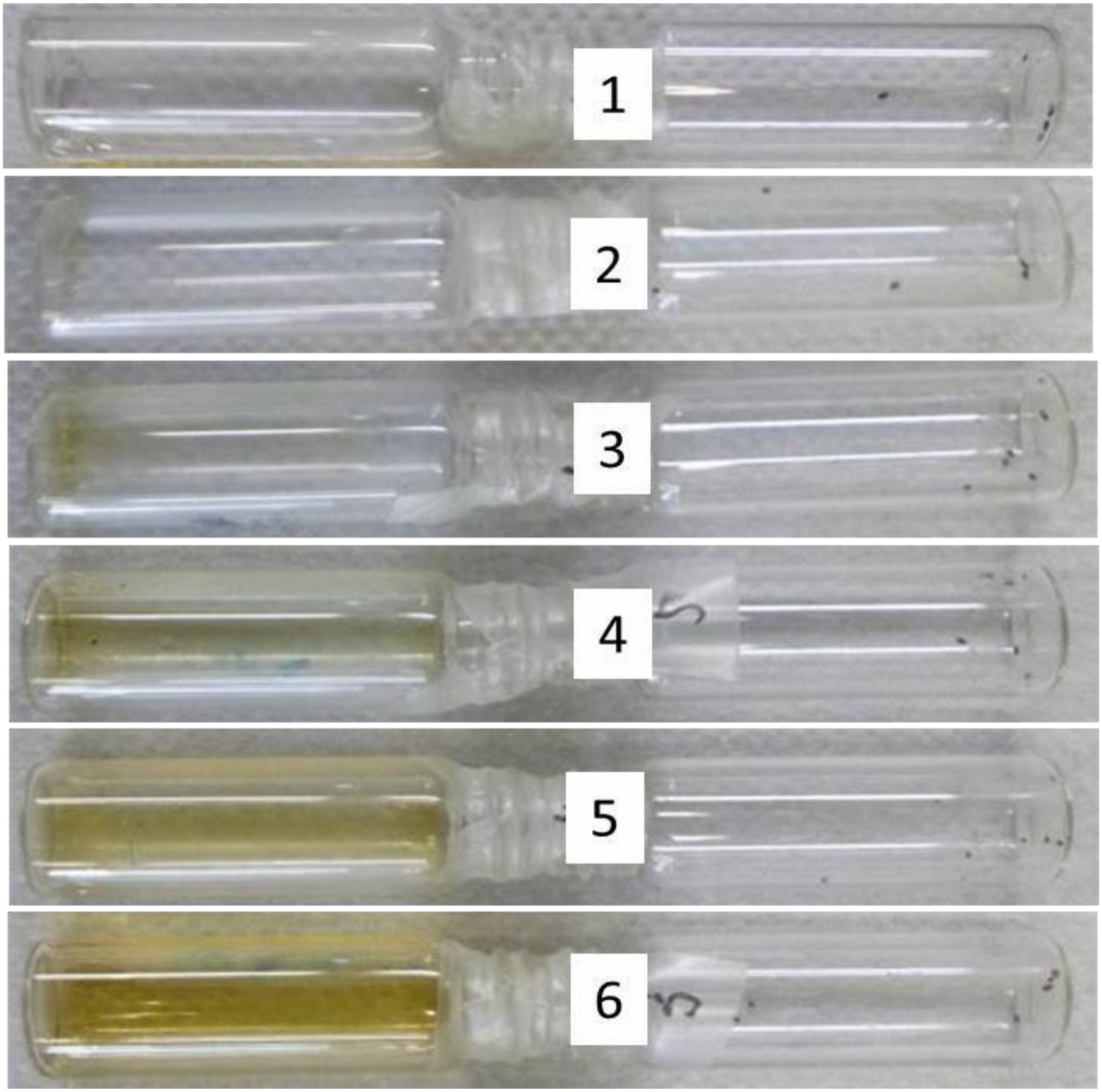

Figure 3. Glass bottles used for estimating the 50% lethal concentration of wood vinegar (WV) for red mites. The bottles on the left were numbered from 1 to 6 and contained 1,800 µl water, 18 µl WV, 180 µl WV, 450 µl WV, 900 µl WV and 1,800 µl WV. Eight red mites were added to each of bottles on the right.

1,800 (bottle 6) µl WV were added to 9 ml glass bottles (Figure 3). Each bottle was connected with another 9 ml glass bottle that included eight red mites, resulting in 18 ml content. The paired glass bottles were kept on a hot plate (37°C) for 18 h, and then the dead red mites counted. The LC_{50} was calculated from the volume of each WV solution and the 18 ml content of the two bottles, and expressed as µl/cm^3. Further, the dead red mites and volume of each WV solution were changed to logarithmic transformation according to Ishimoto and Nagase (2010).

3) Effects of water, rice vinegar, black vinegar, and WV on red mites

The effects of WV on red mites were compared with those of commercial rice vinegar (MizkanJyumaisuKinpu, Mizkan Holdings Co., Ltd., Aichi, Japan) and commercial black

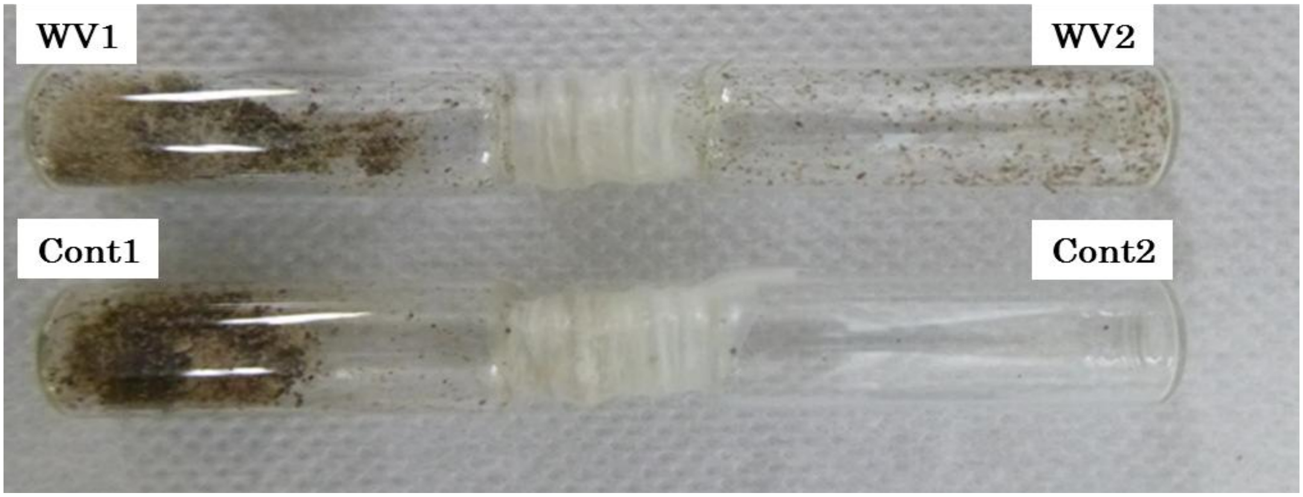

Figure 4. Glass bottles to test the effects of the non-volatilized vinegar (WV) substance on red mites. Only one red mite was observed in Cont2, whereas many red mites were found in the WV 2 bottle after a 48-h exposure to WV.

vinegar (MizkanJungenmaiKurosu, Mizkan Holdings Co., Ltd., Aichi, Japan) which did not include alcohol. Fifteen ml (15 ml) of water, rice vinegar, black vinegar, and WV was added to 628 cm^3 Petri dishes containing filter paper. A cluster of red mites was added to these dishes; the dishes were closed with a lid, and kept in the dark room for 19 h and dead red mites were counted at the end of the incubation.

4) Effect of acetic acid concentration on red mites

As WV includes 2.9% acetic acid (Matsui et al., 1998), acetic acid (CH_3COOH = 60.05, Wako Pure Chemical Osaka, Japan) was diluted from 1 to 10% using water or diluted from 3 to 10% using WV respectively. Each diluted solution was added to a 628 cm^3 Petri dish containing filter paper. Then, a cluster of red mites was added, the dishes were kept in the dark room for 19 h, and the dead red mites counted at the end of the incubation.

5) Effect of non-volatilized WV substances on red mites

A group of red mites found in the dust from a chicken house was placed in a 9 ml glass bottle as a control (Cont 1) (Figure 4). This bottle was connected to another 9 ml empty glass bottle (Cont 2). In the case of the WV experimental group of red mites, a 9 ml glass bottle (WV 2) containing WV solution was dried in the incubator at 40°C for 24 h. This bottle now contained only non-volatilized components of the WV solution. Red mites were then introduced into this bottle, which was then connected to another 9 ml empty glass bottle (WV 2). The mites were incubated in these bottles in total darkness at approximately 30°C (room temperature) for 48 h.

6) Measurement of osmotic pressure and elements of WV

One ml of WV was centrifuged at 15,000 rpm for 5 min. The dark brown sediments included in the liquid and solid parts were separately used for analysis. The osmotic pressure of the liquid part of the sediments and chicken blood were measured using an osmometer (Micro Osmo Master Auto OM-815, Bio Medical Science Inc., Tokyo Japan). In contrast, the solid part of sediments was mixed with 50-µl tap water and some of the solid part of sediments mixed with 50-µl absolute alcohol. These mixtures were diluted to 10% solutions with distilled water, and their osmotic pressures measured. The third solid part of sediments was used to observe ultrastructural and elemental composition by SEM-energy dispersive X-ray spectroscopy (Hitachi Ltd., Tokyo, Japan). Thus, the kinds of elements and their distribution percentage in the WV particles were analyzed.

7) Effects of WV immersion on red mite morphology

The red mites were immersed in water, 500 times-diluted WV, or WV solution overnight. Dead red mites were dried for 1, 24, and 72 h, and their morphological alterations were continuously observed using SEM respectively.

8) Effects of spraying WV on the distribution pattern of WV particles inside red mite bodies *in vivo*

WV was sprayed on red mites in the chicken house and they were collected two weeks later. They were immersed in a chloroform solution for a few seconds, air-dried and then observed using a light microscope.

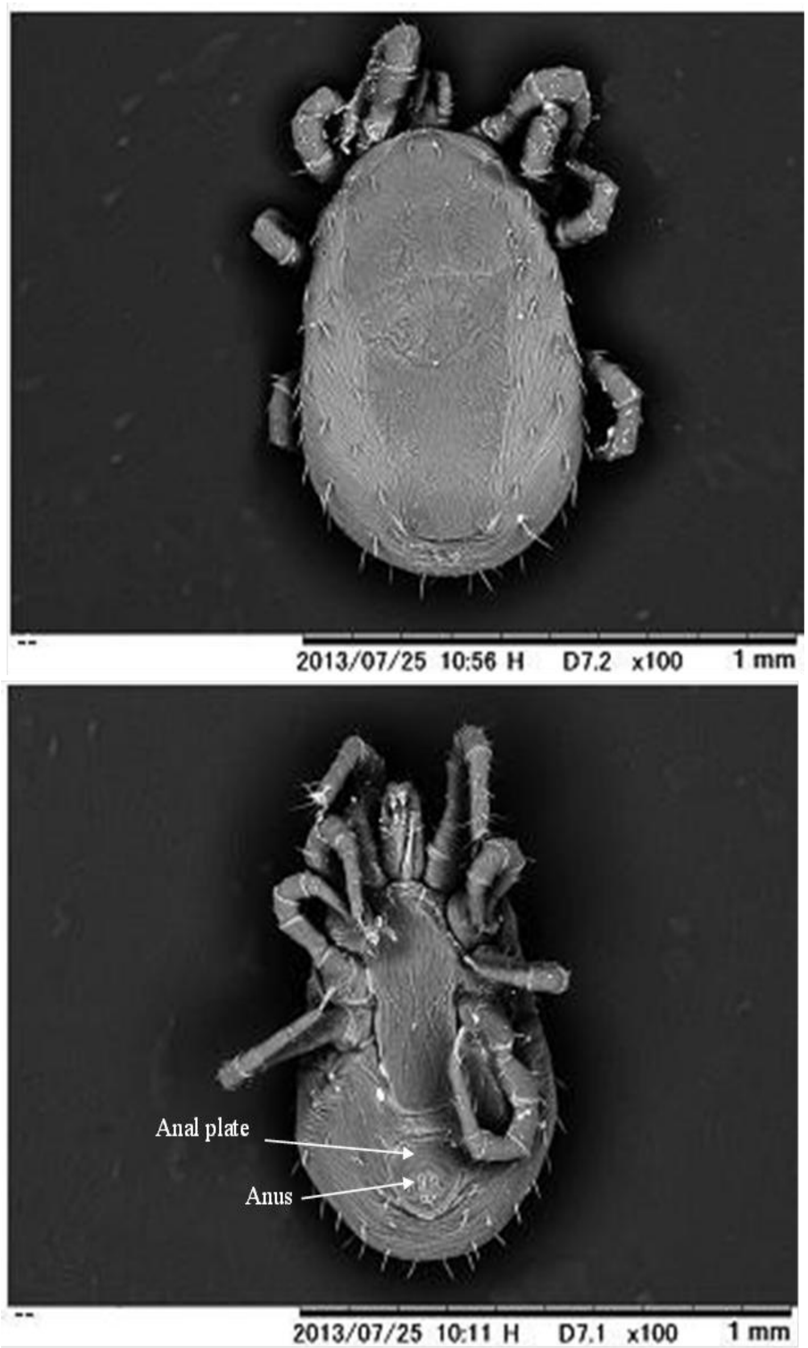

Figure 5. Scanning electron microscopic image of a red mite. Upper, dorsal view. Lower, ventral view. In red mites, the anus stays at the posterior part of the anal plate.

9) Effects of WV on the proto-nymph and eggs of red mites

After a powdered form of the solid sediments of WV was sprinkled on the red mites, morphological alterations of the red mites during the proto-nymph period were photographed, and a video recording was made. Besides, WV was sprayed to eggs of red mites.

RESULTS AND DISCUSSION

1) Identification of red mites by SEM

SEM was a simple and easy machine for identification of red mites. All parasites were identified as red mites, as the anus was positioned at the posterior part of the anal plate (Figure 5) (Nakamae, 2001). According to Kaufman (1996),

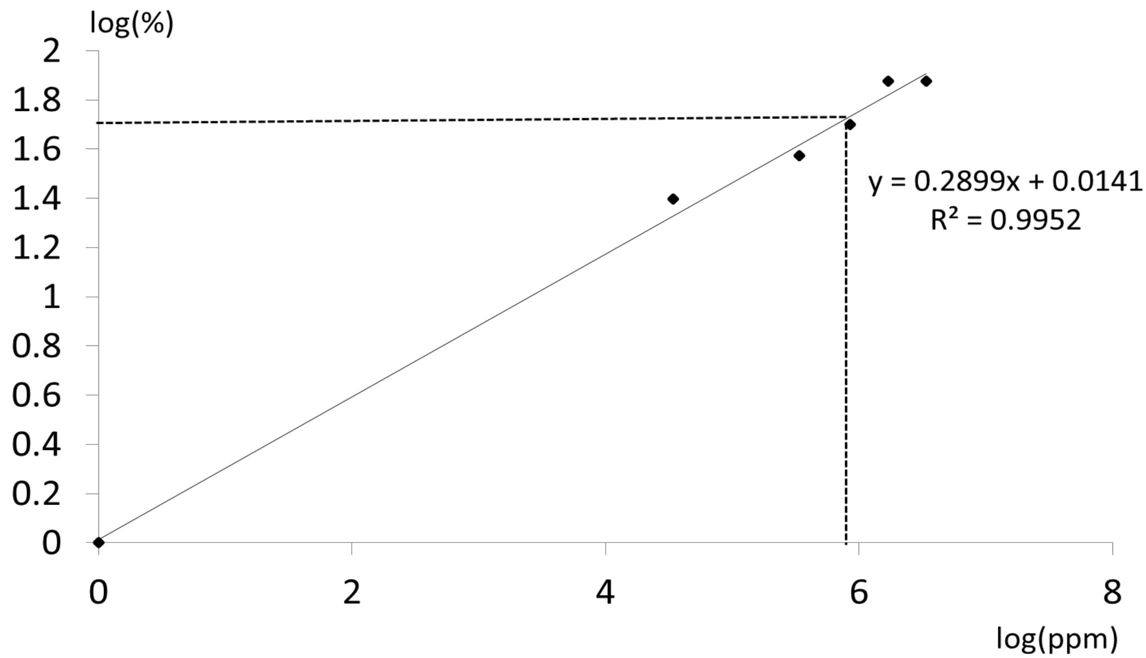

Figure 6. Lethal wood vinegar concentration, 50% (LC_{50}) for red mites. The LC_{50} is estimated to be 18.9 µl/cm^3.

Table 1. Effects of water, rice vinegar, black vinegar and wood vinegar (WV) on the extermination rate of red mites.

Group	Number of red mite		Extermination rate (%)
	Initial	Final dead	
Water	51	12	24
Rice vinegar	68	36	53
Black rice vinegar	42	31	74
WV	38	38	100

WV group shows 100% extermination rate.

the adult female red mite was approximately 1 mm long and 0.4 mm wide, varying in colour from grey to red depending on the degree of engorgement. The red mite in this study was approximately 0.84 mm long and 0.57 mm wide.

2) Estimation of the WV LC_{50} for red mites

The WV LC_{50} value for red mites was calculated from the volume of each WV solution and the 18 ml contents of the two bottles, and was estimated as 18.9 µl/cm^3 (Figure 6).The mortality of red mites to 50 µl/cm^3 WV was approximately 75%, and this percent mortality was maintained at 100 µl/cm^3, suggesting that the volatilized WV gas killed the red mites. To identify whether acetic acid, the main component of WV, was the main factor causing the red mites to escape, mites were placed and covered in Petri dishes containing WV in the next *in vitro* experiment.

3) Effects of water, rice and black vinegars, and WV on red mite extermination rate

Table 1 shows the effects of water, rice vinegar, black vinegar, and WV on red mite extermination rate. Among these vinegars, WV revealed the highest extermination rate (100%) of red mites, which may have occurred because of the volatilized vinegar gas in the sealed dish. As WV includes 2.9% acetic acid (Matsui et al., 1998), acetic acid may have caused the high extermination rate. Thus, acetic acid was diluted to investigate its effect on red mites in the next *in vitro* experiment.

4) Effect of acetic acid concentration on red mites

Figure 7 shows the effects of acetic acid diluted with water or WV on red mite extermination rate. The extermination

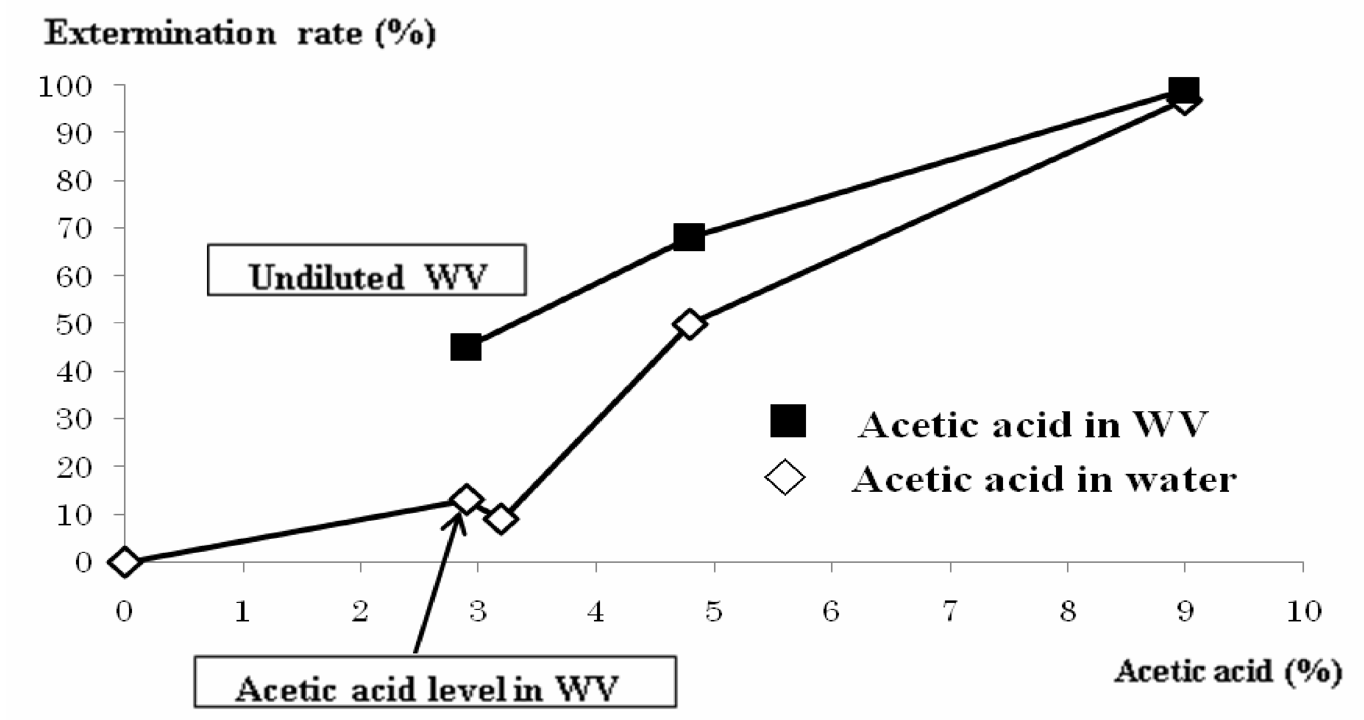

Figure 7. Effect of acetic acid concentration in water or in wood vinegar (WV) on red mites extermination rate. At acetic acid level in undiluted WV, the red mite extermination rate is better in the WV solution, suggesting that WV might have another components to kill red mites.

or WV on red mite extermination rate. The extermination rate of both solvents increased with increasing acetic acid percentage, suggesting that the acetic acid had an extermination effect on the red mites. However, at the 2.9% acetic acid level in water or in WV, the undiluted WV solution included 2.9% acetic acid and other chemical compounds from natural bark at the 2.9% acetic acid level in water or in WV, whereas, the acetic acid diluted with water included only 2.9% chemically produced acetic acid. The red mite extermination rate was better in the former solution than that in the latter solution, suggesting that such an extermination effect might be more associated with other components in WV than with acetic acid. To confirm the possibility that components other than acetic acid in WV conferred an exterminating effect, the following experiment was conducted.

5) Effect of a non-volatilized WV substance on red mites

As shown in Figure 4, at the end of the 48 h exposure to non-volatilized WV substance, only one red mite was observed in the Cont 2 bottle, whereas, many red mites were found in the WV 2 bottle. Because the Cont 1 bottle had no WV, but the WV 2 bottle included non-volatilized WV substance, this result indicates that many of the red mites in the WV 2 bottle escaped from the WV 1 bottle. This result suggests that red mites tried to evade WV. To investigate the main component of WV and underlying mechanism of WV in red mite extermination, osmotic pressure and elemental component of WV were measured.

6) Measurement of WV osmotic pressure and elemental composition

The osmotic pressure values of chicken blood and the liquid part of the sediment collected after centrifuging the WV were 285 and 650 mOsm/L, respectively. When these solvents were diluted to 10% solutions, the values became 6 and 60 mOsm/L, respectively, suggesting that WV has a high osmotic pressure. In contrast, the solid part of the sediment did not easily dissolve in 50 μl tap water (osmotic pressure, 200 mOsm/L), but easily dissolved in 50 μl absolute alcohol (osmotic pressure, 16,000 mOsm/L). After tap water and alcohol were used to prepare the 10% solutions, their osmotic pressures were 23 and 1619 mOsm/L, respectively, suggesting that the WV particles are not water-soluble but lipid-soluble. Figure 8 shows the morphology of some types of the particles (arrows in A), and the distribution of carbon (B) and oxygen (C) elements, and the merged image (D) of these particles by SEM-energy dispersive X-ray spectroscopy. X-ray spectroscopy was used to observe WV particle morphology, analyze the elements comprising these particles, and provided total element composition of the particles in the solid part of the

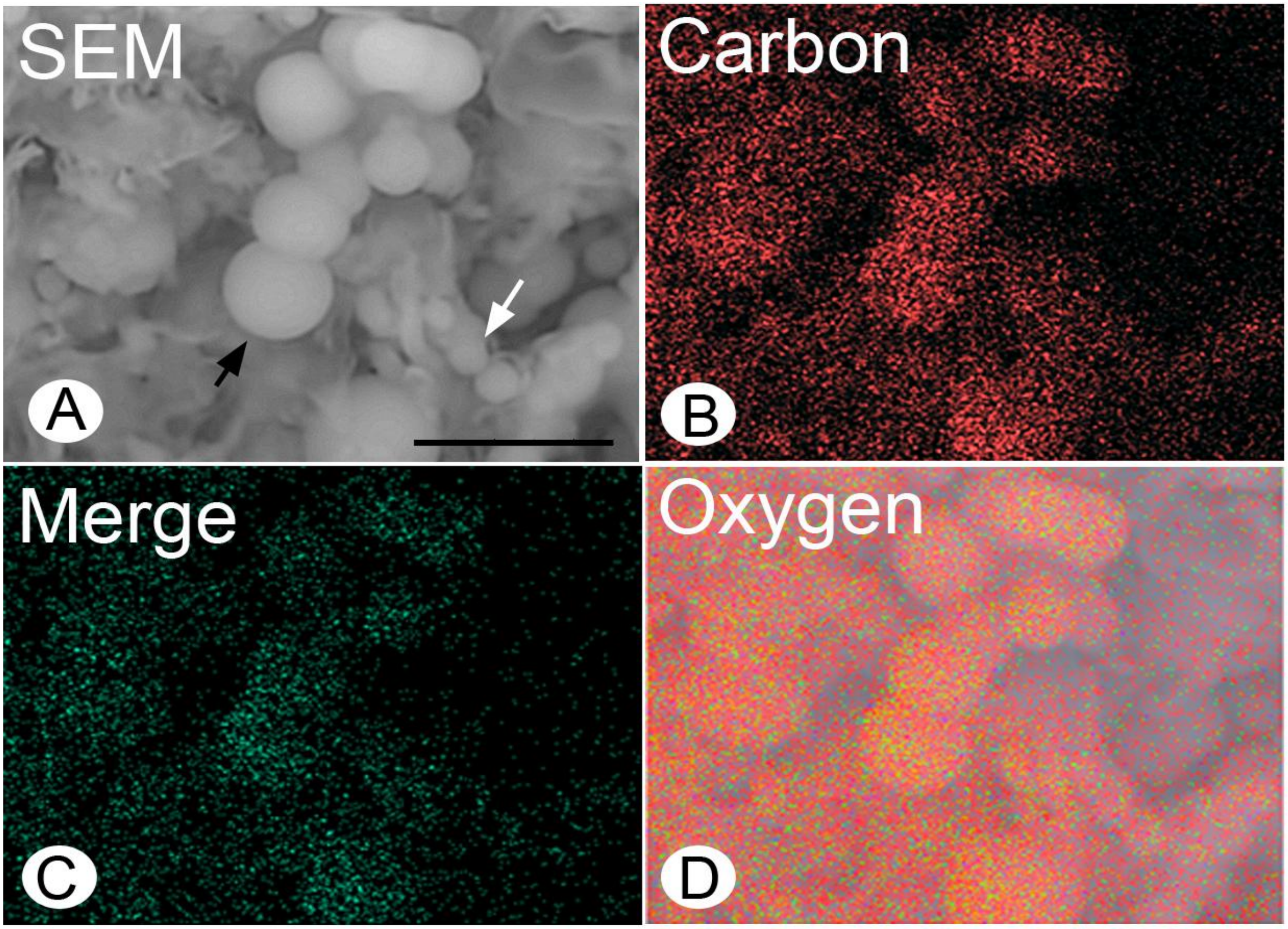

Figure 8. Scanning electron microscopic (SEM) images of some particles (arrows in A), and the distribution of carbon (B), oxygen (C) and their merged image (D) in these particles using SEM–energy dispersive X-ray spectroscopy. Wood vinegar contains many types of particles, including many different elements. Bar: 10 µm.

Table 2. Elemental composition and percentage in wood vinegar.

Elements	Percentage (%)
Carbon	58.154
Oxygen	31.732
Sodium	0.553
Magnesium	0.313
Aluminum	1.750
Silicon	4.796
Potassium	0.318
Calcium	1.334
Iron	0.944
Cobalt	0.015
Nickel	0.026
Gadolinium	0.065

WV sediment (Table 2). These minerals contributed to the osmotic pressure of WV.

An *in vitro* experiment was carried out to clarify how red mite morphology was affected after immersing the red

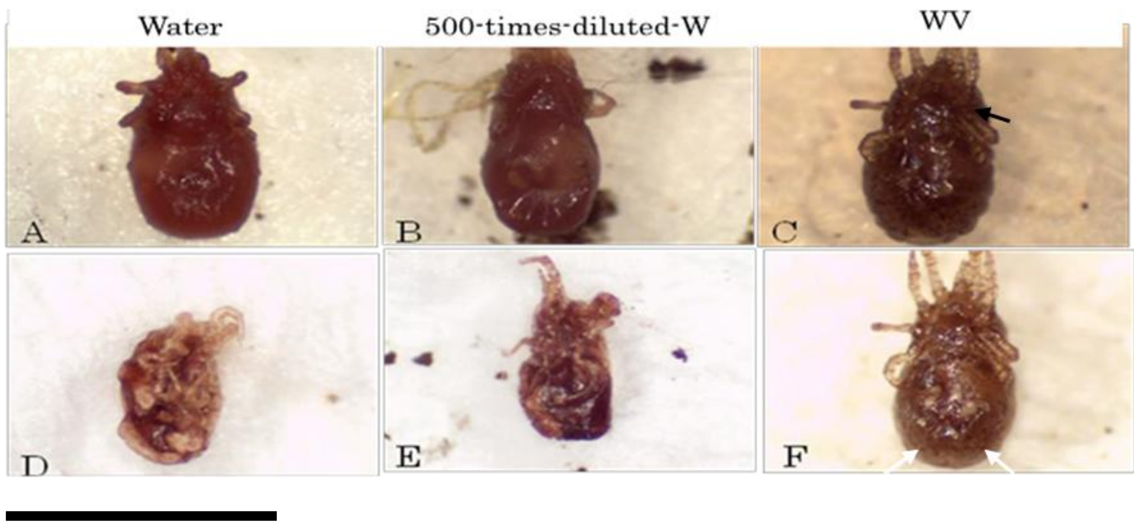

Figure 9. A ventral view of dried red mites at 1 h (upper) and 24 h (lower) after removing them from water (A, D), 500-times- diluted wood vinegar (WV) (B, E) and WV (C, F). The mites immersed in WV do not show extreme shrinkage compared with other groups but are swollen because of WV permeation and included many black particles (arrows). Scale: 1 mm.

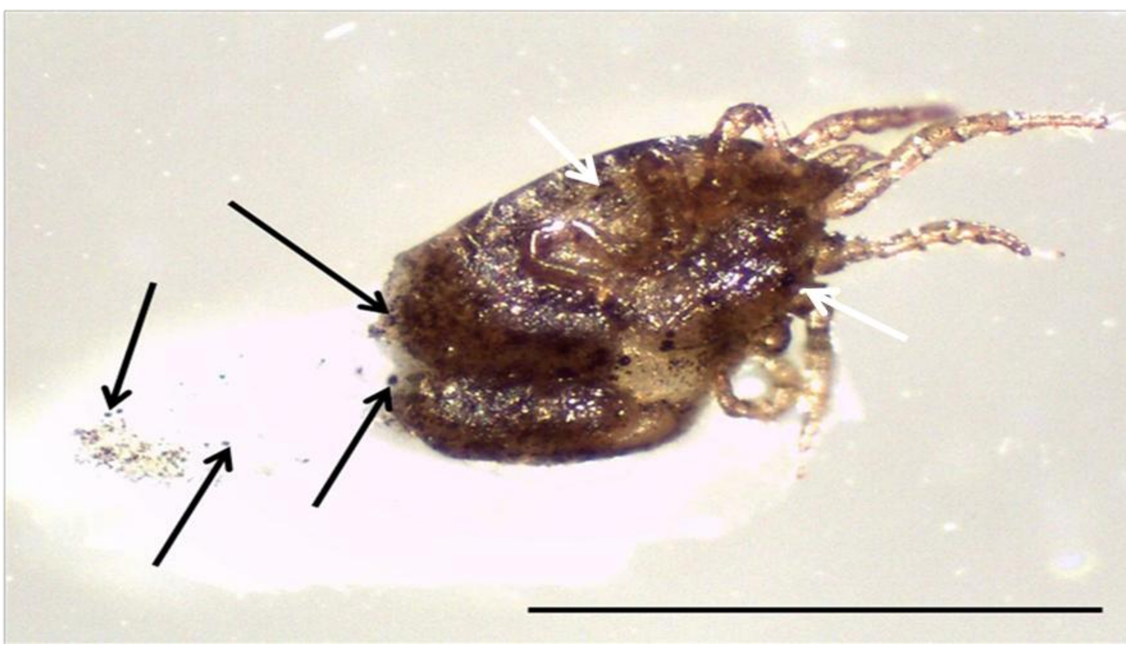

Figure 10. Dried red mites at 72 h after removing them from wood vinegar. Their bodies are swollen because of WV permeation and included many black particles (white arrow). (Scale: 1 mm). Many black particles are exuded (black arrows) after cutting the dorsal body. (Scale: 1 mm).

mites in WV solution.

7) Effects of WV immersion on red mite morphology

The bodies of adult red mites immersed in water and 500-times-diluted WV shrunk, but those immersed in WV swelled (Figure 9). Many black particles were found inside the red mites immersed in WV (arrows in Figures 9C and F). Such swelling and black particles were observed also in drying the mites for 72 h (white arrows in Figure 10). When the dorsal part of the red mite was excised, these black

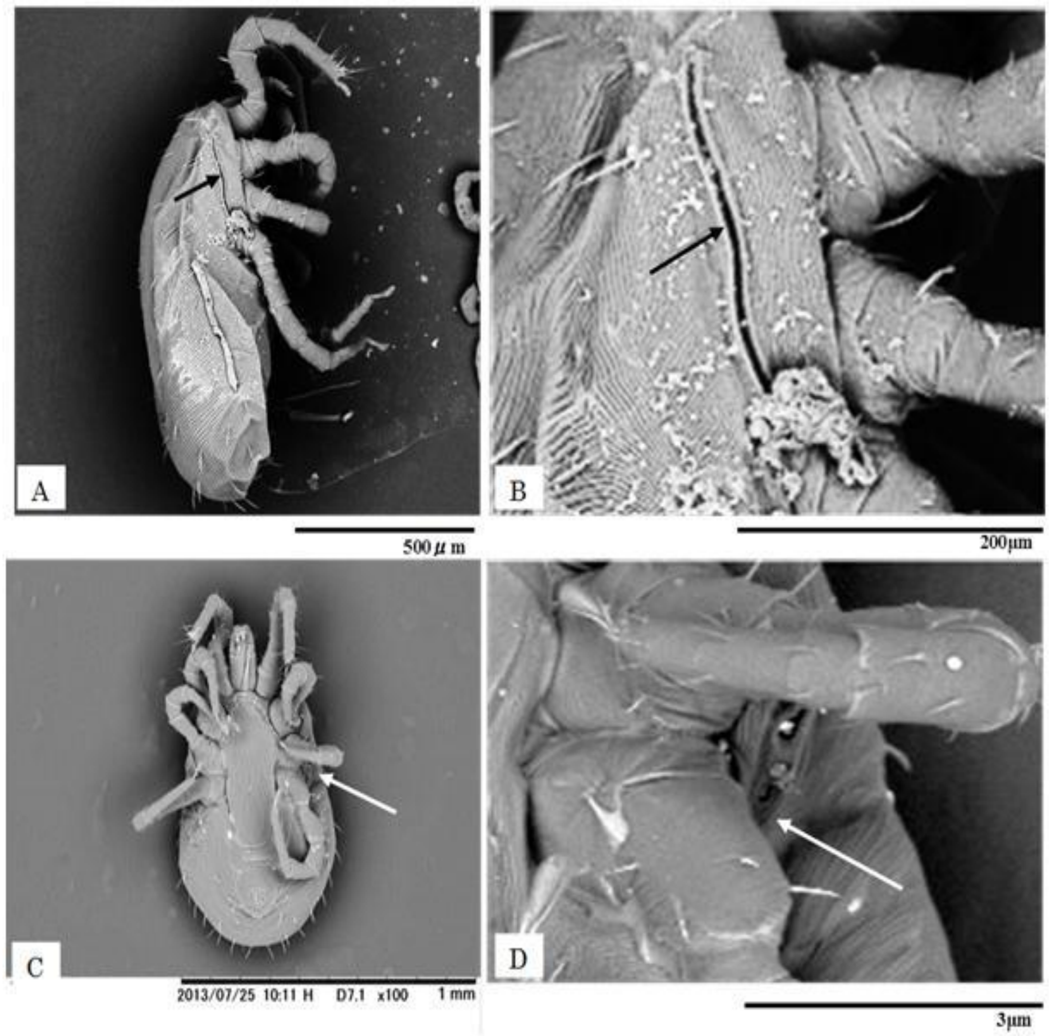

Figure 11. Lateral (A, B) and ventral (C, D) views of red mites (B and D are at higher magnification) as well as spurting body fluid from a proto-nymph body (E). Arrows in A and B indicate peritreme distribution from the first to fourth leg; arrows in C and D indicate the stigma near the fourth leg.

particles came out of the red mite bodies (black arrows in Figure 10). These phenomena seemed to be related to the red mite's respiratory system. The peritreme (long and narrow sulcus) was distributed from the first leg to the fourth leg along the anterior-lateral side of the red mite (arrow in Figures. 11A and B). The stigma (respiratory pores on the insect body) was observed (arrow in Figures. 11C and D) at the end of the peritreme on the fourth leg (Wilson, 1964). When the red mites breathe, WV entered their body from the peritreme and stigma. WV includes many different organic compounds such as alcohol, carboxylic acids, lactones and phenol (Matsui et al., 1998) (Table 3). We could demonstrate many different kinds of minerals in the WV in this study.

It is well known that insects have lectin in their body (Kubo et al., 1991), and that the lectin has a Ca^{2+} dependent hemolytic function (Hatakeyama, 2009). Therefore, the calcium component in the WV mineral matters invaded the body through the respiration system and bound to lectin resulting in swelling of the red mites due to the black particles connected to the lectin. This action of the mite's body fluid may have been related to the high osmotic pressure of WV because of its organic and mineral matter. These mechanisms of WV on adult red mites and young proto-nymph period as well as, on eggs probably occurred in the hen house when the WV was sprayed, because the WV liquid remained around the knots of cages because of its high surface tension. The in vivo experiment was conducted in the hen house.

8) Effects of spraying WV on the distribution pattern of WV particles inside red mite bodies *in vivo*

WV showed a potential for high efficiency in repelling insects and showed the highest significant efficacy of

Table 3. Chemical composition of vinegars from broadleaf tree barks, oak woody parts, cedar woody parts and bamboo.

Group	Compound	Content/mg l^{-1}			
		Barks	Woody part		Bamboo
		Broad-leaf trees	Oak	Cedar	
Alcohol	Methanol	1777	3318	56	2662
	Ethanol	8	13	0	28
Ketone	Acetone	43	670	0	154
Carboxylic acid and lactone	Acetic acid	29290	70046	4302	15224
	Propionic acid	1173	2500	114	649
	Butylic acid and/or	260	1893	76	240
	γ-Butylactone Valeric acid	0	39	12	0
	Crotonic acid	31	40	17	0
	Benzoic acid	33	83	24	28
Furan	Furfural	28	419	0	2151
	5-Methylfurfural	10	99	0	48
	2-Acetylfuran	46	45	0	34
	Furfuryl alcohol	15	0	0	0
	Tetrahydrofurfuryl alcohol	8	20	0	0
Phenol	Phenol	244	323	7	354
	o-Cresol	40	40	0	35
	m-Cresol	73	66	0	38
	p-Cresol	72	38	9	70
Alkyl phenol	4-Ethylphenol and/or 3,5-Xylenol	22	0	0	170
	2,5-Xylenol	14	0	0	3
	2,6-Xylenol	16	9	0	2
Guaiacol	Guaiacol	188	396	18	260
	4-Methylguaiacol	111	111	10	69
	4-Ethylguaiacol	38	29	6	27
	Vanillin	23	25	278	11
	Acetoguaiacone	25	-	-	-
Syringol	Syringol	178	1303	0	209
	4-Methylsyringol	78	14	0	43
	4-Ethylsyringol	30	9	0	42
	4-Propylsyringol	-[a]	58	0	20
	Syringaldehyde	50	-	-	-
	Acetosyringone	44	-	-	-
	Methyl syringate	24	-	-	-
Other compounds	Cyclotene	101	95	67	113
	Maltol	43	346	67	0

a: Not determined.
Citation from Matsui et al., (1998).

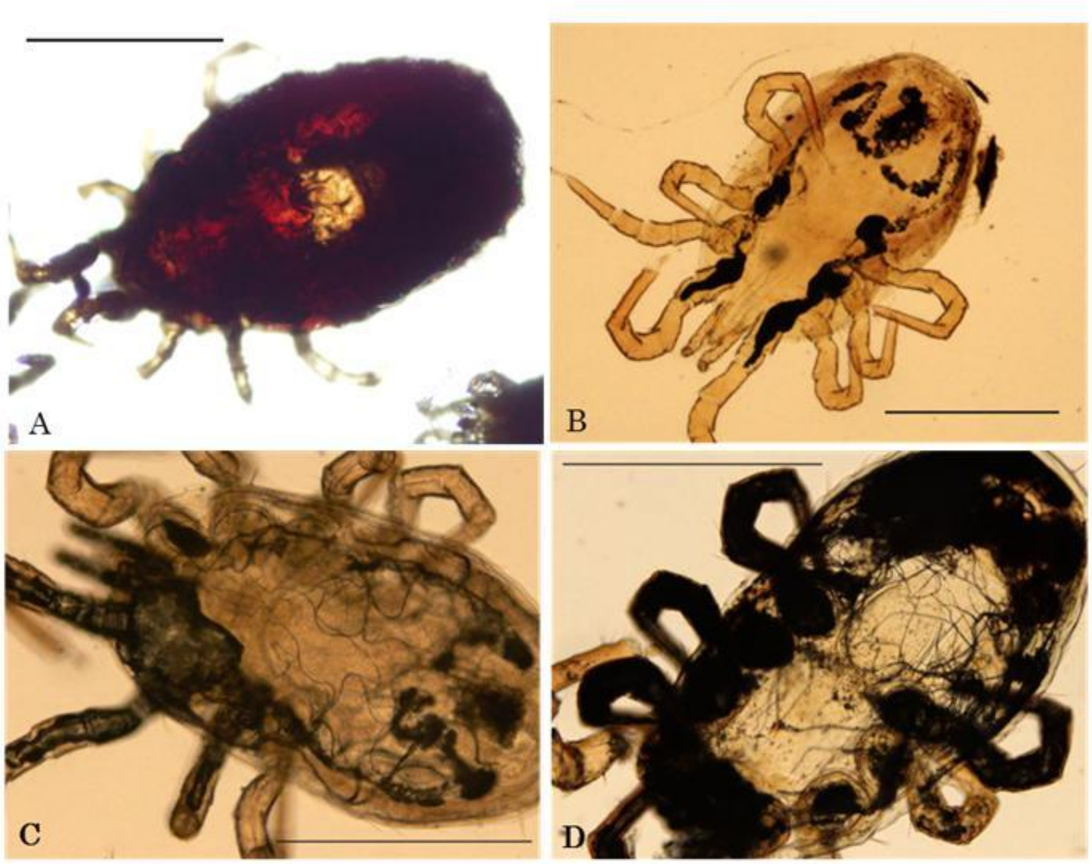

Figure 12. Light microscopy images of red mite revealing sucked blood (A) and red mites two weeks after being sprayed with wood vinegar (B–D). Invaded particles of vinegar from the peritreme (B), mouth (C) and both peritreme and mouth (D) are observed, but blood meal is not found in these mites.

reducing pest infestation in soybean (Pangnakorn et al., 2007, 2010). However, the mechanism of effects of wood vinegar on pest was not cleared. In this study, before spraying WV, sucked blood was observed inside the body of the red mites (Figure 12A). After spraying, red mites immediately disappeared from surface wires of the hen cage. Two weeks after spraying, the mites hidden in the small cracks of the cage were collected; no sucked blood was present even two weeks after spraying but had the same WV particles as those shown in the paragraph of the Effects of WV immersion on red mite morphology'. According to the distribution pattern of WV particles, it is likely that such particles are invaded from the peritreme (Figure 12B), mouth (Figure 12C) or both peritreme and mouth (Figure 12D) when the mites respired. It is estimated that WV contains many minerals such as carbon and oxygen (Table 2) as well as constituents such as acetic acid, methanol and propionic acid (Table 3, Matsui et al., 1998). These characteristic particles may be formed by the low carbonization temperature (300 to 400°C) of broadleaf tree bark because wood charcoal is generally produced by a high carbonization temperature (1,000°C). The present in vivo result that red mites sprayed with WV at the chicken house had WV particles without blood suggests that spraying WV initially has a repellent effect on red mites. In the next stage, WV appears to have an insecticidal effect because these red mites were not sucking blood and as a result die soon while hiding in the small cracks of the cage in the chicken house.

9) Effects of WV on the proto-nymph and eggs of red mites

As the red mite is soft, and only a short peritreme exists near the fourth leg during the proto-nymph (first young insect of red mite) period, a video recording demonstrated that body fluid continuously sprouted from the soft body when the powdered form of solid WV sediment was sprinkled on the red mite proto-nymph (Figure 13A). In the case of eggs of red mites, the inside contents were also exuding from eggs by spraying WV (Figure 13B).

Red mites are the most important problem in large-scale cage feeding systems with high bird densities in the current egg layer breeding method. Using chemicals for red mite control has induced the development of red mite chemical

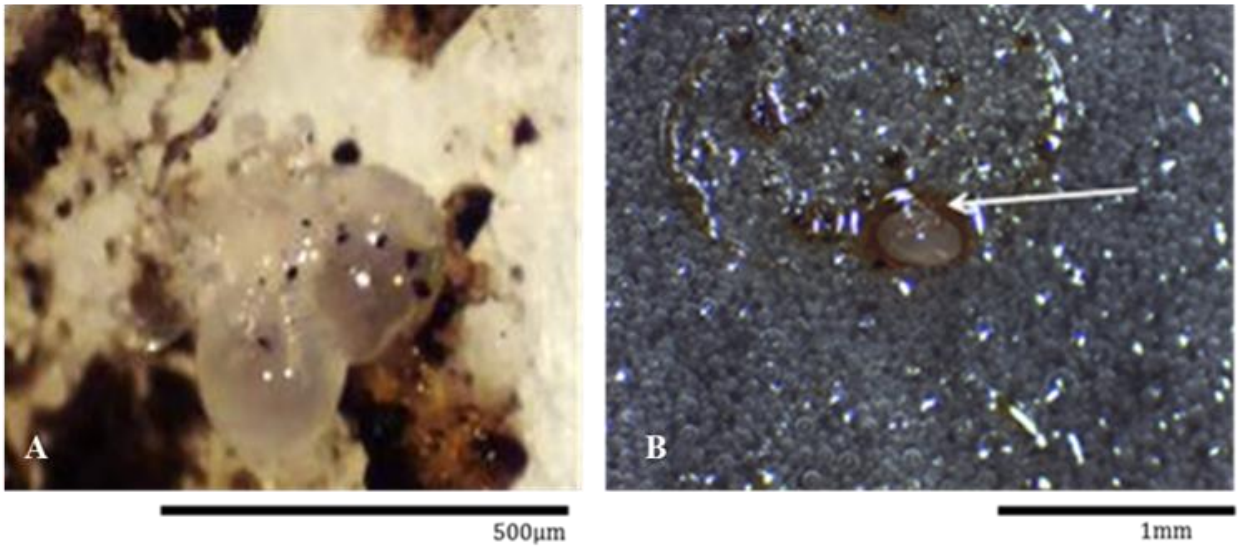

Figure 13. Spurting fluid from a proto-nymph body after sprinkling WV powder (A) and from egg of red mites after spraying WV.

resistance and chemical and antibiotic residues in food and caused environmental contamination (Dalton and Mulcahy, 2001). Furthermore, such chemicals are very expensive. WV is a natural substance residue obtained during wood charcoal preparation, suggesting that WV is safer and less expensive than other chemicals and enable organic farming.

Conclusions

In conclusion, the present *in vitro* and histological studies suggest that the exterminating effect of WV on red mites is induced by the uptake during the respiration of many types of compounds in WV into the peritreme and stigma, resulting in increased osmolality; the $LC_{50}$of WV was 18.9 µl/cm^3. The use of WV contribute to produce high quality farm products without using medicines and to reduce environmental contamination.

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