Exterminating Effect of Wood Vinegar to Red Mites and its Safety to Chickens

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Effects of wood vinegar (WV) on red mites, safety test for chicks, and egg production performance were examined. In the counting of red mite numbers at cage knots using RGB color range of red mites by the image analyzer, the red mite were decreased after spraying ($P<0.01$). In a WV safety test for 8-day-old chicks, one ml of water, original WV, or WV diluted 500 and 1,000 times were tube-fed twice daily for 6 days. The rate of body weight gain was decreased in original WV group than that in the control ($P<0.05$), but it was not different in both WV dilution groups compared with that in the water-only group. In spraying one ml WV to the hen’s face twice per day for 8 days, the egg production performances of WV group did not changed so much compared with those of the control and initial day. These results suggest that the WV did not reduced production performance.

From these results, WV could exterminate red mites, and did not reduce egg production, suggesting that WV is a useful natural substance to exterminate red mites without harmful effect.

Key words: egg production, extermination, laying hen, mortality, red mite, wood vinegar


Introduction

Red mites (Dermapyrus gallinae, poultry red mite, chicken mite) are the most important epidemiological and economical problem for the poultry industry worldwide. This red mite is a blood-sucking pest that causes losses in poultry production because of irritation and anemia. Red mite-infected hens self-groom and scratch their heads during the day and night (Kilpinen et al., 2005). Infected hens also show reduced weight gain (Chauve, 1988; Kilpinen et al., 2005), egg production, and egg quality (Chauve, 1988; Cencek, 2003), as well as anemia and death (Kilpinen et al., 2005). In some case, red mites cause staining of the egg shell surface (Cencek, 2003). Therefore, poultry farmers must clean empty houses between flock cycles with 70°C high-pressure steam to control the red mites. Furthermore, a variety of chemical medicines have been also used. However, continued use of chemicals has induced the development of drug-resistant red mites. Furthermore, chemical use results in environmental contamination. Therefore, alternatives are being developed to control red mites and to reduce environmental pollution through the use of natural resources. Traps impregnated with neem oil originating from the Azadirachta indica tree significantly reduce red mite field populations (Lundh et al., 2005), and pure garlic juice quickly kills these mites (Maurer et al., 2009). These reports suggest that plant-derived compounds may be useful to control red mites.

A wood vinegar (WV) solution was prepared after cooling smoke during charcoal preparation from the bark of broad-leaf trees. As WV includes phenols (Matsui et al., 1998), and inhibits the growth of Salmonella Enteritidis but stimulates the growth of Enterococcus faecium (Watarai and Tana, 2005), we conducted a preliminary test to determine whether WV could kill red mites, resulting in that the red mites disappeared from the chicken cages after spraying 500 dilution WV.

Therefore, purpose of the present work was to establish whether the WV can exterminate red mites without lowering egg production performance by spraying WV on red mites and chickens.

Materials and Methods

Effects of Spraying WV on Red Mites in Cage Knots

Eight points of cage in a hen laying house knots were
randomly selected as observation points (treatment: four points were sprayed with 1ml of 500 dilution WV, control: others were not spraying). To analyze the exterminating effect of WV to red mites, each point was taken a photograph with a digital camera. The numbers of red mites appeared on the screen were estimated by the color range of photograph using the image analyzer (Win Roof, MITANI CORPORATION, Tokyo, Japan). Color range was measured using red, green and blue color code (RGB) (range is 0 to 255), and expressed by dot numbers (one dot was 0.02 mm²). In the case of red mite, 82 to 99 color range of red, 47 to 67 color range of green, and 69 to 82 color range of blue (total 5292 RGB color range) were identified as a color of red mites, and mean size of red mites was 36 dots (0.72 mm²). After 24 h spraying to treatment points, the eight points of cage knots were again taken a photograph, and RGB color ranges were compared between control and treatment groups.

**WV Safety Test on Chicks**

A safety test was performed according to the “chick growth test” on the basis of the safety assessment standard for the feed and evaluation procedure (the 597th 20 consumption life article) from the Ministry of Agriculture, Forestry and Fisheries consumption safety chief on May 18, 2008. Twenty-four Sanuki Cochin male chicks (8 days old) were tube-fed water, original WV, or WV diluted 500 and 1,000 times with water. One ml of each solution was given twice daily in the morning and evening for 6 days. Starter mash diet for layers (CP, 21.0%; ME, 3,000 kcal/kg) was fed *ad libitum*, and initial and final body weights were measured to calculate body weight gain rate (body weight gain/initial body weight). Lethal Dose, 50% (LD₅₀) value was calculated using the formulas:

\[(A/6+B/6) ÷ 2 = C\]

\[2g × 6 days = 12 ÷ C\]

where,

A= initial body weight of six birds

B= final body weight of live birds

C= mean body weight

**Effects of Spraying WV on Egg Production Performance in Laying Hens**

Sixteen hens (Sonia strain, 24-week-old) with similar egg production performance were selected. They were continuously fed the same diets (CP, 17%; ME, 11.93 MJ/kg), and divided into 2 groups: control and WV spraying groups. One ml of original WV solution was sprayed on the hen’s face twice per day for 8 days in WV group (control was only feeding conventional basal diet without spraying). Feed intake, hen-day egg production, egg mass weight, egg weight, feed efficiency, shell-breaking strength, shell thickness, shell ratio, albumin ratio, yolk ratio, yolk color, and Haugh units before spraying WV were compared with those after WV spraying to hens.

**Statistical Analysis**

The numbers of red mites, body weight gain and egg production performance were analyzed by one-way analysis of variance with the Statistical Package for the Social Sciences software version 10.0 for Windows (SPSS, Inc., Chicago, IL, USA). In the RGB color range and production performance, significant differences between groups were analyzed using independent samples t-test. Differences were considered significant at *P*<0.05. In body weight gain, difference among treatment groups was assessed by Duncan’s test and were considered significant at *P*<0.05. The production performance and numbers of red mites between before and after spraying were assessed by a paired samples t-test in SPSS at *P*<0.05.

All experiments and collection protocols in the present study were managed in accordance with the guidelines and rules for animal experiments, Kagawa University, Japan.

**Results**

**Effects of Spraying WV on Red Mites in Cage Knots**

Fig. 1 shows example pictures of cage knots during experiment. Many red mites appeared on cage surfaces from the inner part of cage knots, and many red mite eggs were destroyed at points 1 and 2, but not 3 after spraying the WV. Table 1 shows the RGB color ranges in cage knots of two groups were 29±7 and 59±14, respectively (Table 1; *P*=0.329). Then, one group showing value of 29±7 was not sprayed WV (control); the group of 59±14 was sprayed WV (treatment). The RGB color ranges on the next day was 40±4 in the control group, but 10±2 in the treatment group, resulting in that the RGB color ranges of treatment group decreased than those of the control (*P*<0.01). When these values of 29 and 40 were calculated as an increased ratio (%), it was +37±29 in the control group. This means that the numbers of red mites showed 37% increase in a day (*P*<0.01). In the case of the treatment group, the increased ratio was −91±5; the numbers of red mites showed 91% decrease in a day after spraying WV than those before spraying it (*P*<0.01).

**WV Chick Safety Test**

Two birds in the original WV group were dead on days 4 or 6 after starting the safety test. The rate of body weight gain was significantly lower in the original WV group (*P*<0.05) but was not different in the 500 and 1,000 dilution WV groups compared with that in the water-only group (Table 2). Although there was no systematic attempt to quantify other measurement, no differences were observed in behavior, feather color, or feces among the groups. Calculated LD₅₀ of the original WV group for chicks was 126,316 mg/kg.

**Effects of Spraying WV on Egg Production Performance in Laying Hens**

Table 3 shows the egg production performance of the control (no spraying) and WV group on initial (upper) and final (lower) days for 8-day-raising. Egg production performance was not different between groups both on initial and final days. Compared with values of production performance on the initial day, yolk color value was higher on the final day (*P*<0.05). In the WV group, the albumin ratio (*P*<0.05) was lower, but egg mass weight (*P*<0.05) and shell ratio (*P*<0.01) were higher than those of the initial day.
The current egg layer breeding method has changed from a floor feeding system to a large-scale cage feeding system with high bird densities. This change in feeding system has promoted development of red mites, and stressed birds, resulting in poor production performance. Red mites live in the knots of cages, on the egg conveyor belt, or hidden in cracks and crevices where they lay eggs during the day. Because the feeding-oviposition cycle of the red mite repeats about every third day (Kirkwood, 1963; Desch, 1984), its life cycle can be completed within 1 week, and large populations can be rapidly established in poultry houses (Chirico et al., 2003). Therefore, poultry industry needs an effective insecticide with sustained but not harmful effect.

A variety of chemical medicines such as organo phosphorous compounds (dichlorvos, malathion, metriphonate), carbamates (propoxur), and synthetic pyrethroids (cyfluthrin and pyrethrin I and II) were used in poultry house (Höglund et al., 1995; Chirico and Tauson, 2002; Murano, 2007; Yamauchi et al., 2008). Table 1. Exterminating Effect of Wood Vinegar

<table>
<thead>
<tr>
<th>RGB color range</th>
<th>Control (4)</th>
<th>Treatment (4)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>29±7</td>
<td>59±14</td>
<td>0.329</td>
</tr>
<tr>
<td>After</td>
<td>40±4</td>
<td>10±2</td>
<td>0.005</td>
</tr>
<tr>
<td>Increased ratio (%)</td>
<td>+37±29</td>
<td>−91±5</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Effect of spraying wood vinegar (WV) on red, green and blue (RGB) color range of cage knots

Values are means±SEM of the number of observed points in parentheses.

Fig. 1. Red mites on cage knots before and after wood vinegar (WV) spraying. 1 & 2: WV spraying, 3: no WV spraying.
Murano et al., 2008). However, these reports show that many chemicals were difficult to exterminate red mites perfectly in a short term. The result of spraying to cage knots revealed 91% of red mites was disappeared by the present WV solution after 24h spraying (Table 1). Although there was no systematic attempt to quantify other measurement, many red mite eggs were destroyed and their eggs could not be found at WV spraying points 1 and 2 (Fig. 1). Collectively, these results indicated that WV had strong insecticidal effect, but not evasion effect on the red mites in a short term.

As for the chemical medicines as mentioned above, the toxicity becomes the problem (Kim et al., 2007). Actually, when hens with red mites had soaked and their cages were

![Table 2. Effect of oral administration of wood vinegar (WV) solution on body weight gain and mortality in chicks (mean ± SEM, n=6)](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight gain rate</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>77.8±2.9\textsuperscript{a}</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td>WV</td>
<td>56.3±10.1\textsuperscript{b}</td>
<td>2/6 (33%)</td>
</tr>
<tr>
<td>500 WV</td>
<td>85.8±4.4\textsuperscript{c}</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td>1,000 WV</td>
<td>82.5±4.2\textsuperscript{d}</td>
<td>0/6 (0%)</td>
</tr>
</tbody>
</table>

Means with different letters are significantly different at \( p < 0.05 \).

500 WV: wood vinegar diluted with water at 500 times, 1,000 WV: wood vinegar diluted with water at 1,000 times.

![Table 3. Effect of spraying wood vinegar (WV) on egg production performance in laying hens (mean ± SEM, n=4)](image)

<table>
<thead>
<tr>
<th>Items</th>
<th>Control</th>
<th>WV</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (g/hen/day)</td>
<td>90.4</td>
<td>98.5</td>
<td>—</td>
</tr>
<tr>
<td>Hen-day egg production (%)</td>
<td>86.0±4.0</td>
<td>75.0±9.6</td>
<td>0.129</td>
</tr>
<tr>
<td>Egg mass weight (g/hen/day)</td>
<td>46.0±3.2</td>
<td>36.3±3.8\textsuperscript{a}</td>
<td>0.100</td>
</tr>
<tr>
<td>Egg weight</td>
<td>62.4±2.4</td>
<td>64.2±1.8</td>
<td>0.580</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>0.69</td>
<td>0.65</td>
<td>—</td>
</tr>
<tr>
<td>Shell-breaking strength (kg/cm\textsuperscript{2})</td>
<td>2.68±0.45</td>
<td>2.88±0.22</td>
<td>0.703</td>
</tr>
<tr>
<td>Shell thickness (mm)</td>
<td>0.36±0.21</td>
<td>0.37±0.15</td>
<td>0.562</td>
</tr>
<tr>
<td>Shell ratio (%)</td>
<td>9.9±0.5</td>
<td>10.1±0.3\textsuperscript{a}</td>
<td>0.672</td>
</tr>
<tr>
<td>Albumin ratio (%)</td>
<td>62.9±0.8</td>
<td>62.2±0.7\textsuperscript{a}</td>
<td>0.500</td>
</tr>
<tr>
<td>Yolk ratio (%)</td>
<td>27.2±0.3</td>
<td>27.7±0.4</td>
<td>0.476</td>
</tr>
<tr>
<td>Yolk color</td>
<td>11.8±0.3\textsuperscript{(a)}</td>
<td>12.0±0.00</td>
<td>0.391</td>
</tr>
<tr>
<td>Haugh unit</td>
<td>80.8±3.6</td>
<td>75.1±4.7</td>
<td>0.370</td>
</tr>
</tbody>
</table>

Means with different superscripts differ from upper and lower significantly \( p < 0.05 \).
sprayed every day for two weeks using the custom method (soak; 1,000 times diluted pyrethroid insecticide solution, spraying: a mixed solution of liquid paraffin and pyrethroid insecticide), we found that the treatment could not control the red mites while egg production rate gradually decreased to 30% and birds began to die (unpublished data). According to Poisonous and Deleterious Substances Control Law, a poison has an LD$_{50} < 50$ mg/kg, and deleterious compound has an LD$_{50} < 300$ mg/kg. In rats, the LD$_{50}$ was 5,000 mg/kg (Watanabe et al., 2008). In this study, the present higher value of LD$_{50}$ of 126,316 mg/kg than that of rat was safe for chicks, suggesting that diluted WV has no harmful effect on chickens. Moreover, WV spraying the hen’s face with WV may not have induced stress because their egg production performances did not changed (Table 3). Therefore, it is also possible that WV can improve poultry production. However, the albumin ratio was lower than those of the initial day. Further work is needed to investigate the effect of WV spraying on egg quality.

Although it is considered that they suck blood from hens at night and leave before daybreak (Arends, 1991), they parasitize and propagate on the chickens day and night without leaving the chickens before daybreak (Nakamae et al., 1997). Thus, daytime spraying could be useful for anthelmintic of red mites. In fact, we observed in spraying a 200-ml solution of 500 times diluted WV with tap water to red mites accumulating on hen’s feathers around the cloaca (Left in Fig. 2), the WV could exterminate these red mites on day 4 after spraying (Right in Fig. 2).

Another problem for chemical medicines is the development of drug resistance. Repeated long-term control of red mites with the chemicals induces the development of heritable resistance in the mites (Zeman, 1987). It is likely that the WV might not induce such a resistance in the mites, because the WV is natural substance. In the report using natural substances, cardboard traps containing 20% neem oil from the tree Azadirachta indica induced a 92% reduction of red mites after placing traps at the mites’ aggregation sites for four weeks (Lundh et al., 2005). These reports imply that natural substance such plant can also control the red mites without drug resistance.

In conclusion, WV could exterminate red mites and spraying WV on hens did not reduce egg production, suggesting that WV is a useful natural substance to control red mites without lowering egg production performance.

References


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