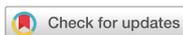


Rapid Communication  
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# Application of carbon dioxide as a novel approach to eradicate poultry red mites

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## ABSTRACT

Poultry red mites (PRMs), *Dermanyssus gallinae*, are one of the most harmful ectoparasites of laying hens. Because of their public health impact, safe, effective methods to eradicate PRMs are greatly needed. Carbon dioxide (CO<sub>2</sub>) was shown to eradicate phytophagous mites; however, there is no evidence that PRMs can be eradicated by CO<sub>2</sub>. Thus, the efficacy of CO<sub>2</sub>, applied by direct-spraying and dry ice-generated exposure, for eradicating PRMs was investigated. Both treatments eradicated > 85% of PRMs within 24 h and 100% of PRMs by 120 h of post-treatment. Therefore, these novel approaches may be useful for eradicating PRMs in clinical settings.

**Keywords:** Acaricides; asphyxiator; carbon dioxide; *Dermanyssus gallinae*

## INTRODUCTION

The poultry red mite (PRM), *Dermanyssus gallinae* (De Geer, 1778), is an important hematophagous ectoparasite that attacks resting hens, mainly at night, for a blood meal [1]. After feeding, the mites hide in cracks and crevices, where they mate and lay eggs. PRM infestations have several negative effects on the hens. Because an adult mite ingests approximately 0.2 µL of blood [2]. The PRM infestation and the mortality of hens are strongly related, and it is mentioned in some reports that the mortality rate of hens increases ten-times following severe infestation [3]. In addition to the direct physiological effects, PRMs may also be carriers of several important disease-causing agents, such as *Salmonella* [4] and the microbial agents that cause spirochetosis [5] and encephalitis.

PRMs are considered to be the most damaging pests in poultry egg production [6-8], and as the number of infections increase, controlling PRMs is becoming very complicated. A range of acaricides, including organophosphates, carbamate, amidine and pyrethroid-based acaricides, are widely used to control PRMs [6-8]. However, many acaricides are not specifically labeled for use against PRMs [6-8]. Fipronil is an acaricide that is authorized for use in plant protection but is not approved for use as a veterinary medicinal product on food-producing animals [9]; however, in many countries, it is illegally used on laying hens to eradicate red mites [9]. Fipronil-contaminated eggs were detected in Belgium in July

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### Conflict of Interest

The authors declare no conflicts of interest.

### Author Contributions

Data curation: Kang J; Formal analysis: Kang J, Park H; Funding acquisition: Kwon YK, Park SW, Kim YS; Investigation: Kang J, Hossain MA, Park H; Methodology: Kang J, Park H, Jeong J; Project administration: Kim JH, Kang MS; Resources: Kang J, Park SW; Software: Kang J, Hossain MA; Supervision: Park SW; Validation: Kang J, Hossain MA, Park H; Visualization: Park SW; Writing - original draft: Hossain MA, Kang J; Writing - review & editing: Hossain MA, Kang J.

2017, which was attributed to the illegal use of fipronil on poultry farms [9]. Furthermore, in various parts of the world, resistance against all classes of acaricides has developed [10].

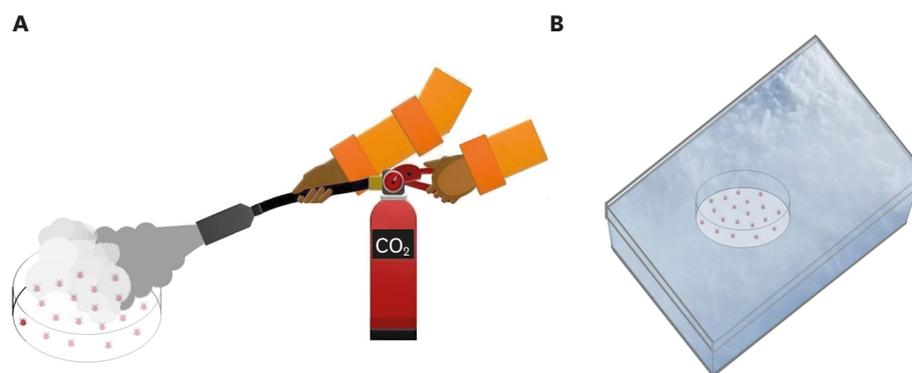
Another important aspect to consider in the development of treatments for PRMs is the safety of non-targets, such as humans, the hosts (hens), and their eggs [10]. In recent years, several alternatives for the safe eradication of PRMs have been developed, including essential oils, biological compounds, predator mites, heat treatments, intermittent lighting programs, inert dusts and even vaccines [10]. It has been reported that the application of carbon dioxide (CO<sub>2</sub>) significantly reduced several species of phytophagous mites by asphyxiation [11]. Therefore, we hypothesized that CO<sub>2</sub> would reduce the viability of PRMs. Therefore, this study was designed to evaluate the efficacy of CO<sub>2</sub> treatment for the eradication of PRMs by exposing PRMs to CO<sub>2</sub> both in a chamber for different durations and by direct spraying.

## MATERIALS AND METHODS

Cardboard traps (100 mm × 70 mm × 3 mm) were prepared in-house using cardboard (Hansol Papertech Company Limited, Korea). To collect mites, cardboard traps were installed at a poultry farm located in Mungyeong city, Republic of Korea as described previously [12]. Then, the traps were collected in plastic zip-lock bags (Perfect Packing Company Limited, China). In one experiment, 20 individual PRMs each were transferred to 4 different petri dishes (SPL Life Sciences Company Limited, Korea) as described previously [13]. Then, CO<sub>2</sub> was sprayed onto the PRMs in petri dishes for 10 sec by using a CO<sub>2</sub> cylinder equipped with a sprayer (Catalina Cylinders, USA). The density of CO<sub>2</sub> in the cylinder was 449,901 ppm or g/m<sup>3</sup>, and the exposure time was 10 sec. Then, the petri dishes were kept in normal experimental condition (25°C ± 2°C, < 65% related humidity) without sealing and the percentage of killed PRMs was determined after 2, 24, 48, and 120 h of CO<sub>2</sub> spraying. The mites were classified as dead if they were in a dorsal position or if no motility was detected by touching with a fine-tipped artist's paintbrush. In another experiment, 3 different amounts (100, 300 and 500 g) of dry ice were used in 3 different chambers (Semadeni Plastics Group, Switzerland) to generate CO<sub>2</sub>. The CO<sub>2</sub> densities in the chambers containing 100, 300, and 500 g of dry ice were 833, 2,500 and 4,167 ppm or g/m<sup>3</sup>, respectively. Twelve petri dishes containing 20 PRMs each were separately placed in 3 different CO<sub>2</sub> chambers (4 petri dishes/chamber) for 1, 2, 5, 10, and 30 min. The densities of CO<sub>2</sub> inside those respective petri dishes were same as in those chambers. After exposing the PRM in CO<sub>2</sub> inside the chambers for the above mentioned duration, those petri dishes were kept in normal experimental condition without sealing. The percentages of killed PRMs after exposing for certain times (1, 2, 5, 10, and 30 min) in the chambers were determined at 4 different times (2, 24, 48, and 120 h) using the procedure described above. Petri dishes that contain 20 PRMs in each were separately kept at normal experimental condition without CO<sub>2</sub> exposing was considered as control, and the mortality rate of control PRM was determined as mentioned for CO<sub>2</sub> treated groups. These experiments were conducted as illustrated in **Fig. 1** and were repeated 3 times.

## RESULTS AND DISCUSSION

The chemicals used to control PRMs may have adverse effects on humans, both directly, when workers are exposed to the chemicals, and indirectly, through the consumption of pesticide residue-containing poultry eggs [14]. Therefore, we sought to develop an effective and



**Fig. 1.** Schematic representations of CO<sub>2</sub> treatment methods to eradicate PRMs in this study. (A) Direct spraying of CO<sub>2</sub>. (B) Exposure to dry ice-generated CO<sub>2</sub> in a closed chamber. CO<sub>2</sub>, carbon dioxide; PRM, poultry red mite.

convenient treatment that eradicates PRMs without causing any harm to chickens or other non-target individuals. In this study, we investigated the efficacy of 2 different CO<sub>2</sub> treatment methods for eradicating PRMs. After CO<sub>2</sub> treatment using either method, the survival rates of the parasites were reduced (**Table 1**) and the mortality rate increased with increased CO<sub>2</sub> exposure time. This suggests that CO<sub>2</sub> may affect one or more vital physiological processes of the PRMs such as respiration [11]. A significant percentage (26%–78%) of the PRMs were killed after 2 h of CO<sub>2</sub> application by both spraying and exposure, and most or all of the remaining mites were killed (73%–100%) within 120 h after CO<sub>2</sub> application. Importantly, the mites did not recover from the CO<sub>2</sub> treatment.

Under normal experimental condition, without any treatment (control), the PRMs remained stable until 24 h of observation; however, by 120 h of observation, 1.7% of red mites dead. In contrast, direct CO<sub>2</sub> spraying resulted in high killing rates (75, 89, 97, and 100%) within 2, 24, 48, and 120 h, respectively. In the CO<sub>2</sub> exposure method, the PRM killing rates were lower when the CO<sub>2</sub> density in the chamber was lower and higher when the CO<sub>2</sub> density was higher. Exposure of the PRMs to both 2,500 and 4,167 ppm CO<sub>2</sub> for ≥ 10 min killed > 75% of the test parasites within 2 h and > 80% of PRMs within 24 h, respectively. The killing rates of PRMs obtained by exposing to both 2,500 and 4,167 ppm CO<sub>2</sub> for 10 min were similar to the killing rate obtained by the spraying method. Moreover, the findings of this study demonstrated that both the CO<sub>2</sub> spraying and exposure methods can completely eradicate PRM. Using the spraying method, complete eradication of the PRMs was observed within 120 h of CO<sub>2</sub> treatment. PRMs exposed to 2500 ppm CO<sub>2</sub> for ≥ 10 min were completely eradicated within 120 h of CO<sub>2</sub> treatment. PRMs exposed to 4,167 ppm CO<sub>2</sub> were also completely eradicated

**Table 1.** Efficacy of CO<sub>2</sub> treatment for the eradication of poultry red mites using different application methods

CO <sub>2</sub> treatment method	Eradication rate (%) at different time intervals after CO <sub>2</sub> treatment											
	2 h			24 h			48 h			120 h		
	833 ppm	2,500 ppm	4,167 ppm	833 ppm	2,500 ppm	4,167 ppm	833 ppm	2,500 ppm	4,167 ppm	833ppm	2,500 ppm	4,167 ppm
Control		0.0 ± 0.0			0.0 ± 0.0			0.0 ± 0.0			1.7 ± 2.0	
Exposure (1 min)	25.8 ± 5.6	34.5 ± 6.5	42.5 ± 4.8	35.2 ± 5.4	41.0 ± 4.3	48.7 ± 10.1	50.5 ± 6.7	55.4 ± 4.3	60.2 ± 9.4	72.6 ± 8.5	92.5 ± 3.2	95.2 ± 5.5
Exposure (2 min)	31.5 ± 9.9	48.7 ± 4.6	50.2 ± 5.8	35.6 ± 4.3	55.5 ± 5.7	59.5 ± 6.7	59.5 ± 10.0	68.7 ± 6.8	69.2 ± 5.6	73.7 ± 6.7	89.2 ± 9.5	97.7 ± 9.0
Exposure (5 min)	45.4 ± 5.7	72.2 ± 5.4	70.7 ± 6.7	55.8 ± 2.6	76.7 ± 6.8	75.5 ± 6.0	57.4 ± 9.7	85.5 ± 7.4	80.6 ± 7.2	85.3 ± 8.4	92.2 ± 8.7	99.5 ± 8.5
Exposure (10 min)	59.7 ± 7.1	77.5 ± 4.3	76.6 ± 4.3	64.5 ± 9.3	80.6 ± 5.4	82.8 ± 4.6	69.5 ± 2.7	85.6 ± 8.1	88.4 ± 11.2	84.2 ± 12.5	95.7 ± 7.5	97.5 ± 8.7
Exposure (30 min)	62.5 ± 5.7	77.8 ± 5.8	77.2 ± 7.3	72.8 ± 10.0	82.2 ± 9.8	85.5 ± 6.3	78.5 ± 6.9	89.8 ± 3.5	91.5 ± 9.7	85.4 ± 11.7	96.2 ± 5.4	99.0 ± 2.0
Spray (10 sec)		74.6 ± 15.2			89.4 ± 11.5			96.8 ± 12.8			100.0 ± 0.0	

Data represent the mean ± SD of 3 replicate analyses. The eradication rates of poultry red mites in CO<sub>2</sub> treatment groups (both CO<sub>2</sub> exposure and spraying) are significantly (*p* < 0.05) different than the control group at each observation time (2, 24, 48, and 129 h). CO<sub>2</sub>, carbon dioxide.

within 120 h of CO<sub>2</sub> treatment, regardless of the duration of CO<sub>2</sub> exposure. It is also clearly evident that the PRM killing rate was dependent on the CO<sub>2</sub> density in the chamber. The killing trends for both methods were nearly the same. Furthermore, ≥ 80% mortality in 24 h is normally considered to be sufficiently effective according to the “Guideline for testing efficacy of insecticide for prevention of infectious diseases (Ministry of Food and Drug safety of Korea)” [15]. Based on this guideline, the spraying of CO<sub>2</sub> for 10 sec is effective enough to eradicate red mites. Similarly, exposing the PRMs in CO<sub>2</sub> for at least 10 min confirm the efficacy of this treatment method.

To completely eradicate PRMs (100%) in a closed environment, like a chicken room on a farm, CO<sub>2</sub> exposure in a sealed room for 30 min may be a preferable treatment method. The CO<sub>2</sub> spraying method can be applied to birds to eradicate PRMs before introducing them to a farm. Moreover, spraying CO<sub>2</sub> in local environments where PRM colonies are located, including walls, floors, roosts, nests, boxes, cracks, and crevices, may lead to their rapid eradication. PRMs can be completely eradicated from chicken rooms by exposing the rooms to CO<sub>2</sub> under closed conditions at a time when they are “all in all out.” These novel PRM eradication methods can be further optimized for application in clinical settings. For this purpose, a spray bottle or can of CO<sub>2</sub> could be used for rapid application and ease of transport. However, further studies are needed to determine the specific mechanisms underlying the eradication of PRMs by CO<sub>2</sub>.

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