The Use of Ozone for Controlling European House Dust Mite, *Dermatophagoides pteronyssinus* (Trouessart)

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Abstract

Hypersensitivity allergies are mostly caused by house dust mite (HDM), *Dermatophagoides pteronyssinus* (Trouessart). Common control methods for HDM include disposal of bedding sets and fabrics, vacuuming, washing, using plant extracts and applying chemicals. Hence, the application of ozone is a new alternative way to control HDM. The objectives of this experiment were to determine the efficacy of ozone as an HDM killer and to evaluate the allergic levels that remained after the application of ozone. The fumigation method was performed in a laboratory using ozone at the concentrations of 20, 30, 40 and 50 mg l^{-1} in glass chambers (1 m^3) at 1, 2 and 3 h fumigation intervals. The mortality percentages of HDM were observed at 24 h after the treatment. The allergic levels appearing in supernatants were analyzed by an enzyme-linked immunosorbent assay (ELISA) method. Ozone fumigations of 30 mg l^{-1} concentration for 3 h completely killed HDM. In addition, ozone fumigations of $\geq$ 40 mg l^{-1} for 3 h reduced the amount of allergen by >50%, which was a significantly higher reduction than seen at 20-30 mg l^{-1} (35.8-45.8%). The study suggests the ozone fumigation at 30 mg l^{-1} concentration treated for at least 3 h, 40 mg l^{-1} for 2 h, or 50 mg l^{-1} for 1 h could be used as a new alternative method to control HDM.

Keywords: Acari, Pyroglyphidae, *Dermatophagoides pteronyssinis*, ELISA, fumigation, allergies, ozone, *Der p1*
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1. Introduction

House dust mites (HDM) are classified as members of Arachnida and are related to ticks. They are small in size and typically of 0.2-0.3 mm in length. HDM have translucent bodies under the microscope. The principal HDM species such as *Dermatophagoides pteronyssinus* (Trouessart), *Dermatophagoides farina* (Hughes), *Euroglyphus maynei* (Cooreman) belong to the family Pyroglyphidae, whereas *Blomia tropicalis* (Bronswijk) belongs to Glycyphagidae. The pyroglyphid species are at the top in terms of global frequency and abundance [1, 2]. Remarkably, they are the most common allergy-causing mites found in houses worldwide [3]. Insung *et al.* [4] investigated

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the species diversity of HDM in central Thailand and the most abundant species found was *D. pteronyssinus* (69.94%). The incidence of atopic diseases has shown to be increasing all over the world. Allergic rhinitis affects about 10-25% of the population and up to 40% of children, and its incidence is increasing worldwide [5, 6]. In Thailand, the incidence of asthma and allergic rhinitis was 51.4% [7]. The most common sensitizing allergens are HDM. *Dermatophagoides pteronyssinus* and *D. farinae* were major HDM species that were found to be associated with respiratory allergic diseases [7]. In general, sensitization due to house dust mite allergens (HDMAs) appeared in 65 to 130 million people around the world, which is about 50% of the total number of asthmatic patients globally [8]. Recent reports suggested that HDMAs commonly caused allergic diseases found in humans, dogs and cats [9]. Trakultivakorn and Nuglor [10] revealed that *D. pteronyssinus* was one of the major sources of allergy and *Der p1* and *Der p2* were important mite allergens found in Chiang Mai, Thailand.

With the elimination of HDM, the source of HDMAs affecting allergic patients should be reduced. There are many methods of killing HDM; chemical methods such as application of acaricides and physical measures such as heating, freezing and washing of bedding and fabric. However, in the case of the application of acaricides onto carpets, mattresses or furniture, users can come into direct contact with the chemical acaricides. Thus, the use of acaricides is not recommended for controlling HMD. Theoretically, freezing and heating methods should be effective, but no report has demonstrated benefits from such interventions. HDMAs are also effectively removed by washing of bedding and clothing. By this method, mites are killed by drowning [11]. Vinyl fabric is an option for preventing allergic patients coming into contact with HDM. However, further investigation on the efficacy of various kinds and qualities of vinyl fabrics and patient satisfaction with vinyl fabric bed encasing has been recommended [12]. Another alternative method used to control HDM effectively is the application of cloves and cinnamon-based essential oil formulas [13, 14]. Moreover, essential oils from cloves and cinnamon were also extremely effective in reducing HDMAs levels [15-17]. However, there are still some limitations on the use of essential oils. One negative is that some users are not satisfied with the smell of essential oils. Thus, the study of a new appropriate method to control HDM is required in order to address the problems in current methods mentioned above.

Molecules of ozone (O_3) are very unstable and can convert into O_2 rapidly by releasing a single oxygen atom that is extremely reactive [18]. This single oxygen atom displays its reactivity when it comes into contact with the cell membranes of bacteria, viruses and fungal or mycotoxin contaminants. It attacks cellular components and disturbs normal cellular activity [18, 19]. When this free oxygen derived from ozone contacts a volatile organic compound, it can remove the odor of the compound [19]. It can destroy cell membranes or protoplasm, disrupting the cellular reactivation of bacteria, coliforms, viruses and protozoa [20]. Ozone application at low concentrations can effectively reduce microbial populations [21]. To eliminate odors, tastes and color occurring in food or water, ozone treatments are commonly used [22]. Furthermore, a high concentration of ozone for fumigation is a qualified method to control various stored product insects, such as *Sitophilus* spp., *Rhyzopertha dominica*, *Plodia interpunctella*, *Galleria mellonella* and *Tribolium confusum* [19, 23-26]. Therefore, there is a possibility to use ozone to eliminate HDM where the actual area affected requires the use of a fumigation method. In addition, it is also worthwhile to assess its effectiveness in reducing HDMAs. Thus, the objectives of this research were to determine the fumigant toxicity of ozone to house dust mites (*Dermatophagoides pteronyssinus*) and to evaluate its effectiveness in reducing house dust mite allergen (*Der p1* allergen).

2. Materials and Methods

2.1 Mite stock culture

Dermatophagoides pteronyssinus was maintained in mite bottles that were modified from 50 ml cell culture flasks and covered with filter paper. The mite bottles were kept in a mite chamber made from an acrylic sheet (size 30×30×50 cm). Saturated KCl was used to control humidity inside the chamber. The mites were fed on a culture medium with the composition modified from Insung and Boczek [27], i.e. a mixture of rat food, wheat germ and yeast with the ratio of 4:4:1 (by weight). The HDM stock culture was kept at 25±1ºC and 86±1% relative humidity in the laboratory of the Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang (KMITL), Thailand.

2.2 Toxicity of ozone fumigation against HDM

Ten adult HDM were introduced into acrylic mite cages adapted from Insung and Boczek [27]. Filter paper was used to close the base holes and glass covers were fixed at the top with wax. All mite cages were placed into a 1 m^3 fumigant glass chamber adapted from Pumnuan *et al*. [28]. Ozone was produced by an ozone generator provided by P.S.C. Trading and Development Co., Ltd., Thailand. The fumigation bioassay was performed by ozone release into the chamber, with the different concentrations of 0 (no ozone), 20, 30, 40 and 50 mg l^{-1}. The fumigation periods were 1, 2 and 3 h, and mortalities of HDM were observed at 24 h thereafter. The death of the mites was confirmed by observing non-movement of their legs when probed with a small hairbrush. The actual death rates were calculated according to Abbot's formula [29]. The completely randomized design (CRD) was applied with five replicates. The percentage of mortality was recorded for each time interval. Lethal concentration (LC_{50} and LC_{90}) and lethal time (LT_{50} and LT_{90}) were determined by the probit method. The analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT) were performed for data analysis.

2.3 Reduction effect to HDMAs

House dust was collected from 20 mattresses that had been used for more than 10 years from different households in Bangkok, Thailand. Afterwards, the house dust was sifted through a fine filtered cloth to sort out the coarse dust. One-gram portions of the house dust were put into petri dishes (10 cm diameter) without a lid. The petri dishes with the house dust material were placed in the 1 m^3 fumigant glass chamber and fumigated with ozone at various concentrations and exposure times following the same method that was used in the fumigation toxicity test against HDM. Then, 0.1 g of material from each treatment was put in a microtube together with 2 ml of phosphate-buffered saline (pH 7) mixed with Tween-20, and the microtubes were placed in a rotator at 4ºC. After 24 h, the contents of the microtubes were homogenized at 2,500 rpm for 15 min. Finally, 1 ml of the supernatant from each tube was removed and kept at 4ºC for further enzyme-linked immunosorbent assay (ELISA). The preparation of house dust material for ELISA test was adapted from Insung *et al*. [15].

2.4 Enzyme-linked immunosorbent assay of the *Der p1* allergen

An enzyme-linked immunosorbent assay (ELISA) was undertaken using the method described by Insung *et al*. [15]. The amount of *Der p1* in the supernatant was measured by an ELISA method using mouse monoclonal antibodies (Indoor Biotechnologies). Three replicates were performed for

each experiment compared with the control group (untreated house dust + spent mite medium). The percentage reductions in the amount of HDMAs were statistically analyzed by applying analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT). The reduction in the amount of *Der p1* was calculated by the following formula:

$$\text{Reduction rate (\%)} = \frac{(\text{Amount of allergen in control} - \text{Amount of allergen in treatment dish}) \text{x} 100\%}{\text{Amount of allergen in control}}$$

3. Results and Discussions

The application of ozone as a fumigant to control HDM in the laboratory at various concentrations and exposure times showed that 3 h fumigation of ozone at ≥ 30 mg l^{-1} completely killed HDM, which was a result of significant difference to 3 h fumigation of ozone at 20 mg l^{-1} , which only reduced HDM by 90.2%. Ozone at 40 mg l^{-1} for 2 h killed HDM by 92.7%, a result which was significantly higher than that for ozone at 30 mg l^{-1} application (68.4%). In addition, the fumigation with 50 mg l^{-1} for 1 h was significantly more effective at killing HDM (81.1%) than was fumigation at 20-40 mg l^{-1}. The appropriate concentration and time of ozone fumigation was 40 mg l^{-1} and 2 h. The LT_{50} and LT_{90} values of ozone fumigation against HDM at 30 mg l^{-1} were 1.30 and 2.35 h, respectively, and 0.99 and 1.67 h, respectively, for 40 mg l^{-1} (Table 1). The result also suggested that ozone fumigation at 20 mg l^{-1} concentration for 3 h was the most effective condition for killing HDM with more than 90% killed, while 1 and 2 h fumigation times presented LC_{50} at 29.98 and 22.96 mg l^{-1}, respectively, and presented LC_{90} at 55.28 and 38.27 mg l^{-1}, respectively. The acaricidal test revealed that ozone fumigation with high concentrations at different periods resulted in a probit regression coefficient (slope) of toxicity regression equation which was higher than that for ozone fumigation at lower concentrations. The concentrations of 40, 30 and 20 mg l^{-1} produced slopes of regression equations with values of 1.788, 1.226 and 0.916, respectively. The slight increment of fumigation time in high ozone concentration resulted in higher HDM mortality rates compared with lower ozone concentration. Likewise, when a longer time of ozone fumigation was used at different concentrations, there was a higher slope of regression equation than for shorter ozone fumigation time. The slopes of regression equations observed were 0.084 and 0.051 at ozone fumigation times at 2 and 1 h, respectively. The result emphasized that a slight increment of ozone concentration used at long fumigation time resulting in higher HDM mortality rate compared with lower ozone fumigation time.

The house dust mite allergen (HDMA), in terms of *Der p1* allergen, was analyzed by the ELISA method. Ozone fumigation at 40 and 50 mg l^{-1} concentration for 3 h reduced the amount of HDMA by 55.4 and 60.3%, results which were not significantly different from each other, but significantly higher than the reduction seen for 20-30 mg l^{-1} (35.8-45.8%). However, ozone application at ≥30 mg l^{-1} with exposure time henceforth 2 h and at 50 mg l^{-1} with exposure time henceforth 1 h resulted in reduction of HDMA by >40%. In contrast, the concentration of ozone at 20 mg l^{-1} with exposure times for 1-3 h reduced the HDMA by only 25.5-35.8%. It was noteworthy that the concentrations of ozone fumigation at 40 and 50 mg l^{-1} resulted in reduction rates of HDMA that were not significantly different, and this was also seen for 30 and 40 mg l^{-1} for 2-3 h fumigation times. All concentrations of ozone fumigation (20-50 mg l^{-1}) for the 2 h fumigation time showed reduction rates of HDMA in the range 34.6-49.2%, with no significant differences among them (Table 2). This study suggests the ozone fumigation at 30 mg l^{-1} concentration treated for at least 3 h or 40 mg l^{-1} for 2 h or 50 mg l^{-1} for 1 h could be used as a new alternative method to control HDM. Those conditions were extremely effective in killing HDM at more than the 80% level, without

significant differences between them and would also reduce the amount of HDMA by >45% effectively.

Table 1. Fumigation toxicities of ozone against house dust mite (*Dermatophagoides pteronyssinus*) (Trouessart) at various concentrations and exposure times

Concentration of ozone (mg l^{-1})	Mortality percentages (means ± S.D.)[1/] Fumigation times			Regression equation	LT_{50} (h)	LT_{90} (h)	Chi-square
	1 h	2 h	3 h				
0 (control)	0.00^{Ea}	0.00^{Da}	0.00^{Ca}	-	-	-	-
20	43.7 ± 3.7^{Db}	49.4 ± 14.1^{Cb}	90.2 ± 6.5^{Ba}	Y=-1.544+0.916x	1.69	3.09	27.99
30	53.0 ± 6.6^{Cb}	68.4 ± 15.8^{Bb}	100.0 ± 0.0^{Aa}	Y=-1.594+1.226x	1.30	2.35	30.15
40	65.4 ± 6.0^{Bc}	92.7 ± 4.3^{Ab}	100.0 ± 0.0^{Aa}	Y=-1.707+1.788x	0.99	1.67	16.51
50	81.1 ± 6.1^{Ab}	97.8 ± 1.8^{Aa}	100.0 ± 0.0^{Aa}	-	-	-	-
Regression equation	Y= -1.518+0.051x	Y= -1.922+0.084x	-	-	-	-	-
LC_{50} (mg l^{-1})	29.98	22.96	-	-	-	-	-
LC_{90} (mg l^{-1})	55.28	38.27	-	-	-	-	-
Chi-square	16.874	7.96	-	-	-	-	-

[1/] Means in column with the same time followed by the same capital letter and means in row followed by the same common letter are not significantly different at the 5% level as determined by DMRT ($P < 0.05$).

Table 2. Percentage reduction of house dust mite (*Dermatophagoides pteronyssinus*) (Trouessart) allergen (*Der p1*) after ozone fumigation at various concentrations and exposure times

Concentration of ozone (mg l^{-1})	Reduction rate (%) (means ± S.D.)[1/] Fumigation times		
	1 h	2 h	3 h
0 (control)	0.00 ± 14.2^{Ca}	0.00 ± 14.2^{Ba}	0.00 ± 14.2^{Da}
20	25.5 ± 3.7^{Ba}	34.6 ± 7.8^{Aa}	35.8 ± 3.2^{Ca}
30	30.0 ± 6.3^{Bb}	40.7 ± 3.3^{Aa}	45.8 ± 3.6^{BCa}
40	39.7 ± 6.3^{Aba}	45.5 ± 3.1^{Aa}	55.4 ± 4.1^{ABa}
50	45.6 ± 1.0^{Ac}	49.2 ± 1.9^{Ab}	60.3 ± 0.2^{Aa}

[1/] Means in column with the same time followed by the same capital letters and means in row followed by the same common letter are not significantly different at the 5% level as determined by DMRT ($P < 0.05$).

Ozone fumigation is a new alternative method for elimination of HDM in households or in accommodation. This study indicates that ozone at 40 mg l^{-1} concentration with 3 h fumigation time could be used to completely control HDM and reduce the amount of HDMA by >50%. This concentration of ozone could also successfully reduce microbial populations. There are many previous reports regarding the efficacy of ozone against many organisms. One finding was that 10 ppm of ozone could remove up to 99% of bacteria and viruses in 10 min. The action of ozone on viruses, even at lower ozone concentrations, compared well to its activity against bacteria at higher concentrations. This is due to the fact that the bacterial wall is more complex than the viral envelope. It was demonstrated that ozone can kill fungi that initiate many human diseases by its oxidizing action, which results in an irreversible cellular effect [20]. Microbial contamination in fresh dates

was able to be completely controlled by ozone application at 5 ppm for at least 1 h [21]. Ozone at <30 ppm concentration was effective in eliminating aflatoxin in contaminated maize feed [19]. Application of 5 ppm ozone showed morphological and mycotoxin inhibition effects on cultures of *Aspergillus flavus* and *Fusarium moniliforme* [30]. Many studies of the control of insect pests in stored products by the use of ozone have been published. Hansen *et al*. [25] reported that ozone fumigation at 135 ppm for 8 days completely killed the young stages of *Sitophilus* spp. and *Rhyzopertha dominica*, while fumigation at 35 ppm for 6 days was a highly effective way to control the adult stages of the insects. The study of Keivanloo *et al*. [26] indicated that the concentrations and exposure times of ozone resulted in different mortality rates of each insect stage. The larval stages were often very much more susceptible than other stages. For example, 5 ppm ozone applied to 12-day-old *Plodia interpunctella* larvae for 2 h produced a 58% mortality rate, and longer exposure times caused higher mortality. However, both egg and pupal stages were more resistant. Continuous ozone treatment at concentration of 13.9 ppm for 2 h was able to kill completely almost all developmental stages of *Ephestia kuehniella* and *Tribolium confusum* [23]. High concentrations of ozone were used to control adult bed bugs with short periods of ozone exposure. When 1800 ppm ozone were applied to bed bugs for 150 min, or 80 ppm applied for 48 h, 100% bed bug mortality resulted [31]. The use of ozone to kill HDM may then also have the further effect of controlling microbial populations, insect pests and other disturbing organisms.

Another good result of the use of ozone for controlling HDM is its direct effect in reducing house dust mite allergen. This study showed that 40 mg l^{-1} ozone fumigation for 3 h completely killed HDM and reduced the amount of HDMA by >50%. Since the allergen is classified as a protein, the characterization of allergens based on a proteomic approach has been introduced [32]. This ability of ozone to reduce the amount of HDMA mentioned above is not surprising in view of Cataldo's work, which shows that ozone causes denaturation of proteins [33]. Besides, the report of Sahab *et al*. [34] indicated that application of 40 ppm ozone for 10 min induced the degradation of aflatoxin by 94%. It also resulted in a reduction in fat and protein content that reached 10.56 and 4.58%, respectively. It is important to note that when ozone fumigation is to take place in the field, care must be taken to avoid its leakage, which could poison organisms outside the desired area. However, in general, ozone decomposes expeditiously. Recommendations for research further to this work could include the study of the insecticidal properties of ozone against other insects, especially insect pests of medical importance such as bed bugs, mosquitoes and cockroaches. The research suggest that ozone fumigation applied at appropriate concentrations could control a number of disturbing organisms effectively.

4. Conclusions

The application of ozone fumigation is a new alternative way for controlling house dust mite. In this experiment, the efficacy of ozone fumigation against HDM and its influence on the reduction of allergen levels were determined. The results indicated that ozone fumigation at concentrations of ≥ 30 mg l^{-1} for 3 h completely killed HDM. In addition, the conditions of 50 mg l^{-1} of ozone fumigation for 1 h, 40 mg l^{-1} for 2 h and 30 mg l^{-1} for 3 h reduced the HDM level by more than 80% and reduced the amount of allergen by more than 45%. This study suggests that ozone fumigation at 30, 40 and 50 mg l^{-1} for at least 3, 2 and 1 h, respectively, could be used as a new alternative methods to control HDM.

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