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Mortality of *Platydema waterhousei* exposed to carbon dioxide and nitrogen atmospheres

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ABSTRACT: *Platydema waterhousei* is a beetle that infests the economically important Rishi mushroom. The toxic effects of four controlled atmospheres to each of the life stages of *Platydema waterhousei* were investigated. The atmospheres used were 100, 80, 60, and 0% CO_2 with N_2 making up the remainder. The atmosphere containing 80% CO_2 was more toxic to eggs than pure CO_2 and N_2. Pure CO_2 was the most lethal to larvae, pupae, and adults. Pupae were the most resistant life stage to all treated gases. Adults were more sensitive than eggs and larvae in pure CO_2 and N_2 atmospheres. Adults as well as eggs were more susceptible than larvae when they were exposed to 60% CO_2. Eggs were more sensitive than adults and larvae when they were held under 80% CO_2. The best control of all life stages could be achieved by exposing to 80% CO_2 for 3 days or to 100% CO_2 for 4 days.

KEYWORDS: *Ganoderma lucidum*, Ling-Zhi mushroom, toxicity, controlled atmosphere, lethal time

INTRODUCTION

Ganoderma lucidum (Fr.) Karsten, commonly known as Rishi or Ling-Zhi mushroom, is a member of the family Polyporaceae. Its medicinal properties have been recognized for many centuries in China and other Asian countries. This fungus has been used as a traditional Chinese medicine to treat virtually all kinds of diseases for over 4000 years[1,2]. As a result of its claimed pharmaceutical values, this mushroom fetches a high price in Thailand. During storage Ling-Zhi mushrooms can be infested with many mycetophagous beetles including *Platydema waterhousei* Gelbien (Coleoptera: Tenebrionidae). Damages caused by *P. waterhousei* include both quantitative loss due to feeding and qualitative loss due to contamination with its body parts, faeces and fibre. Such infestation may make the mushroom totally inedible. The biology of *P. waterhousei* was previously reported by Visarathanonth et al[3].

Disinfestation of stored products is typically achieved by fumigants or pesticides. Such methods are harmful to both user and consumer health, and are environmentally threatening. In addition, consumers nowadays expect a foodstuff which is free of pesticide or with diminished residue levels[4]. Hence, the present trend of pest control is moving towards ecologically friendly alternatives such as modified and controlled atmospheres. These procedures are potential alternatives for post-harvest commodities because they do not leave chemical residues on the food. The effectiveness of modified and controlled atmosphere treatments against stored product insects and other pests has been extensively documented[5,6]. The main difference between these two methods has been described[7]. With controlled atmospheres, gas concentrations are precisely controlled within certain limits by adding nitrogen and/or carbon dioxide. The controlled atmospheres that are lethal to insects usually contain $>$ 20% CO_2 and/or $<$ 1% O_2[5–7]. The information on mechanism of controlled atmospheres on insects is rather limited. Recently, a comprehensive review with respect to this area[7] provides an update on earlier discussions[5,6,8–10]. The effects of controlled

atmospheres on insect pests are remarkably different, depending on the species (strain[11], developmental stage and age within species), the physical factor (temperature and humidity), and on the type and composition of gas in the controlled atmospheres[5–8,12].

The objective of this research was to evaluate the effect of CO_2 and N_2 atmospheres on the mortality of four life stages of *P. waterhousei*. Data from this study will be used to develop an effective control of *P. waterhousei*.

MATERIALS AND METHODS

Insects

Test insects, *P. waterhousei*, were reared on dried Ling-Zhi mushroom, *G. lucidum*, G_2 strain. The mushroom was cut into pieces 1 mm thick and 6.15 ± 0.16 cm long. It was disinfested by heating at 50 °C for 5 h, then cooled to room temperature before using for insect rearing. Ten pairs of newly emerged adults were kept in petri dishes (9 cm diameter) for mating, one pair per dish. Each dish was provided with 3 g of dried Ling-Zhi mushroom as adult food. Six to eight days after mating, females started laying eggs on, or nearby, the mushroom. Adults were transferred to new petri dishes daily. Eggs were collected from 15–30 days old mated females. Eggs were kept 6–7 days until hatching. Newly hatched larvae were reared on the infested mushroom until pupation. Newly emerged adults were transferred to new petri dishes every day. In all experiments, insect cultures were kept at 25.7 ± 4.9 °C, 56.5 ± 11.6% relative humidity and under natural photoperiod, unless otherwise stated.

Exposure to CO_2 and N_2 atmospheres

A preliminary test for four developmental stages of *P. waterhousei* was carried out to find the range of exposure time that caused 0–100% mortality. The preliminary tests were conducted without any replication. For each stage, 25 insects per treatment were transferred to the exposure chamber with 5 g of Ling-Zhi mushroom. The treatments consisted of 4-day-old eggs attached to mushroom, 3–5-day-old 5th instar larvae, 3–5-day-old pupae, and 10–20-day-old adults. Prior to conducting the experiment, the insects were kept at in an exposure chamber for 24 h. This allowed them to adjust to any stress caused by handling and changing temperature[13]. The percentage mortality of each stage was corrected by Abbott's formula[14]. These results were used to determine the optimal ranges of exposure time for the main experiments.

The four controlled atmospheres to which the insects exposed were mixtures of CO_2 and N_2; the CO_2 content of the atmospheres was 100%, 80%, 60%, and 0%. In each case the CO_2 and N_2 from pressurized cylinders were passed through the gas-mixing apparatus (MULTIVAC series KM 100-3MEV). The proportion of a gas combination was regulated by gas-mixing valves. The gas mixtures continuously flowed through the test chambers via the gas incoming tube while the gas inside the chamber flowed out via the gas exit tube. The gases were adjusted to flow at the pressure of 15 psi for 15 min. The gas mixtures in the effluent tubes were then sampled. The concentrations of CO_2 and N_2 were determined by an oxygen and carbon dioxide analyser (Servomex series 1400). The exposure chambers were removed from the gas-mixing apparatus by closing the gas inlet and outlet tubes with Hoffman clamps. After the desired exposure period, the test chambers were opened for 1 h allowing normal air to replace the gases within the chambers. They were then covered with absorbent paper and kept at the conditions explained earlier. Mortality of treated adults was observed one day after exposure. Adults that did not move while prodding were presumed dead. Larval and pupal mortality occurred at 15 and 30 days later, respectively. Larvae that failed to pupate and pupae that were unable to emerge were considered dead. Fifteen days after exposure, egg mortality occurred. Eggs that did not hatch were considered dead. Insects exposed to normal air served as controls.

The main experiments aimed to evaluate the lethal time (LT) for the eggs, larvae, pupae, and adults when exposed to each type of atmosphere. The design for these experiments was a randomized complete block design. The experiments were undertaken by adjusting the exposure times resulting from the preliminary tests. Each experiment was repeated three times with different insect generations. For each replication, 25 insects were placed in each of twenty exposure chambers containing 5 g of mushroom. Four test chambers were exposed to particular atmospheres. Subsequently, each atmospheric chamber was maintained at four exposure times. Thus, 16 chambers were prepared. Four unexposed chambers served as untreated controls. After a desired exposure period, the egg, larval, pupal, and adult mortalities were determined.

Statistical analysis

The LT_{50} and LT_{99}, the times required to kill 50 and 99% of each designated stage, were estimated by probit analysis using the computer program developed by Raymond[15]. The LT_{50} values were compared

to determine the susceptibility of each stage when exposed to particular atmospheres and to determine the relative toxicity of atmospheric types for each stage. The LT_{99} values were examined to discover the exposure times for maximal control of eggs, larvae, pupae, and adults when exposed to each atmospheric type[11].

RESULTS AND DISCUSSION

Effect of controlled atmospheres on mortality

The egg mortality, observed after 4-h exposure to 100% CO_2, was evident by the shrinkage of the eggs. Similarly, egg abnormality was observed when eggs were exposed to other controlled atmospheres for a particular time. The cause of insect death under controlled atmospheres was not investigated in this study. The larval mortality, after exposing to 100% CO_2 as well as to other gas combinations for 6 h, was apparent from the dried body. The pupal mortality, post treatment to 100% CO_2 for 12 h, was characterized by its shriveling and a defectively emerged adult. Pupal abnormality and mortality were also visible when they were tested in other atmospheres. The adult mortality, after exposing to 100% CO_2 for 2 h, was distinguished by its dehydrated body presumably because of rapid loss of water. Similar characteristics were observed when adults were exposed to other atmospheres. Aliniazee[16] reported morphological aberrance in pupae as well as in emerged adults when they were treated with 100% CO_2. For example, emerged adults were wingless, remarkably small, unevenly pigmented and sometimes with partially pupal traits.

The relative toxicity of CO_2 and N_2 atmospheres to four life stages of *P. waterhousei*

The LT_{50} and LT_{99} values are presented in Table 1. The LT_{50} values for eggs, larvae, pupae, and adults are suitable to compare susceptibility between stages and toxicity among atmospheric types because the 95% confidence bands at the LT_{50} are much narrower than for the LT_{99}[11]. The LT_{99} values indicated the time required to produce maximal mortality (99%) for four life stages.

The LT_{50} values for eggs (Table 1) indicate that the 80% CO_2 atmosphere was more lethal to eggs than other atmospheres. Childs and Overby[17] indicated that an atmosphere of 65% CO_2 was more toxic to *L. serricorne* eggs than an atmosphere of 35 or 92% CO_2. Conversely, Rameshbabu et al[18] reported that a 90.4% CO_2 atmosphere was more toxic to *Cryptolestes ferrugineus* eggs than 79 or 68.9% CO_2 atmospheres.

Table 1 The LT_{50} and LT_{99} values (95% confidence limits) for four life stages of *P. waterhousei* when exposed to CO_2 and N_2 atmospheres at 25.7 ± 4.9 °C, 56.5 ± 11.6% relative humidity and under natural photoperiod.

Stage	CO_2 (%)	LT_{50} (h)	LT_{99} (h)
Eggs	100	0.88 (0.23–1.50)	90.4 (15.5–)
	80	0.51 (0.06–0.89)	38.3 (9.06–)
	60	1.05 (0.01–2.27)	∼663 (32–)
	0	1.21 (0.00–5.57)	∼981 (37–)
Larvae	100	2.10 (1.62–2.62)	12.5 (7.9–30.9)
	80	2.46 (1.90–3.12)	17.0 (9.96–50.6)
	60	2.76 (2.26–3.33)	11.2 (7.8–21.7)
	0	2.75 (2.14–3.52)	19.2 (11.0–61.0)
Pupae	100	6.74 (4.78–9.18)	67.2 (27.1–3847)
	80	6.87 (5.20–9.01)	53.2 (24.7–869)
	60	7.91 (5.94–12.76)	88.6 (31.4–)
	0	7.22 (5.72–9.36)	46.8 (23.6–423)
Adults	100	0.67 (0.45–0.83)	3.75 (2.42–10.3)
	80	0.80 (0.57–1.00)	5.25 (3.12–18.5)
	60	1.00 (0.69–1.33)	11.4 (4.9–164)
	0	0.90 (0.52–1.23)	14.5 (5.35–699)

The LT_{50} values for larvae (Table 1) show that pure CO_2 atmosphere was the most toxic to larvae. Similarly, a high percentage of CO_2 was found to be more lethal to *T. castaneum* larvae than a low percentage of CO_2[19].

The LT_{50} values for pupae (Table 1) show that 100% CO_2 was the most deleterious to pupae. Likewise, Mbata et al[20] found that the gas mixture of 99% CO_2:1% O_2 was more lethal to *Callosobruchus subinnotatus* pupae than 60% CO_2:32% N_2: 8% O_2 or 99% N_2:1% O_2.

The LT_{50} values for adults (Table 1) indicate that an atmosphere of 100% CO_2 was the most toxic to adults. Aliniazee[16] reported that 100% CO_2 was more lethal to *T. confusum* and *T. castaneum* adults than 45–80% CO_2. In addition, 92% CO_2 atmosphere was more fatal to *L. serricorne* larvae and adults than 35% CO_2 atmosphere[17]. Therefore, susceptibility of insects to controlled atmospheres was dependent on the type of atmospheres, the insect species, and the developmental stage.

Susceptibility of the four life stages

By comparing the LT_{50} values, the relative sensitivity of *P. waterhousei* to 100% CO_2, in decreasing order, was adults > eggs > larvae > pupae. A similar result was observed when all stages of *P. waterhousei* were exposed to 100% N_2. Likewise, Aliniazee[16] reported that adults of *T. castaneum* and *T. confusum*

were more susceptible than other stages when exposed to 100% CO_2 atmospheres at 15.6, 21.1, and 26.7 °C. The susceptibility of *P. waterhousei* to 60% CO_2, in descending order, was adults $\sim$ eggs $>$ larvae $>$ pupae. In contrast, when all stages of *P. waterhousei* were exposed to 80% CO_2, the eggs were the most sensitive followed by adults, larvae, and pupae.

Maximal mortality

Times required for the maximal mortality of all life stages of *P. waterhousei* are presented in Table 1. According to the shortest exposure time, 38.3 h, 80% CO_2 was clearly the best atmosphere for maximal control of *P. waterhousei* eggs. Shorter exposure times were required for 99% mortality of *C. subinnotatus*[21] and *O. surinamensis*[22] eggs. In contrast, the longer exposure times for 99% lethality were reported for the eggs of *T. castaneum*[19] and *C. chinensis*[23].

The most effective gas combination for maximal control of *P. waterhousei* larvae was 60% CO_2, because of its shortest exposure time. Shorter exposure time for 99% mortality of *O. surinamensis* larvae was required[22]. Longer exposure times for 99% mortality were reported for the larvae of *T. castaneum*[19], *Stegobium paniceum*, and *L. serricorne*[24].

Based on the shortest exposure time, 100% N_2 was the best atmosphere for maximal control of pupae. Longer exposure times for 99% pupal mortality were reported for *C. chinensis*[23], *S. paniceum* and *L. serricorne*[24] and *Sitophilus oryzae*[25]. In contrast, shorter exposure times were needed for 99% pupal mortality of *T. castaneum*[19] and *O. surinamensis*[22]. In all developmental stages, the LT_{99} values decreased with increasing CO_2 concentrations[22].

The 100% CO_2 was suitable for the maximal control of *P. waterhousei* adults because its LT_{99} was the lowest. Shorter exposure time was used for 99% control of *O. surinamensis* adults[22]. Longer exposure times were needed for the complete control of adults for *T. castaneum* and *T. confusum*[16,19,26], and *C. subinnotatus*[21].

In practice, the maximal control of *P. waterhousei* can be achieved by exposing infested Ling-Zhi mushroom in 80% CO_2 for 3 days. In addition, the highest level of disinfestation can be accomplished by exposing to 100% CO_2 with a longer exposure time of 4 days. Moreover, the cost and benefit of selected methods for controlling storage insect pests should be considered. Although the effectiveness of both atmospheres was comparable, the 100% CO_2 is more practical for controlling this insect because this atmospheric type requires only one gas. Thus it is easier to handle and its application costs less because it does not need gas mixing apparatus. This pest control procedure leaves no toxic residue and hence it is safe for consumers and the environment.

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