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The effects of temperature, moisture content, and cricket frass on gas emissions and survival rates in cricket farming at Honghee Village, Kalasin Province, Thailand

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Abstract

Cricket frass accumulation in ponds presents a waste management challenge that can impact cricket farming productivity and quality. This study examines the effects of temperature, moisture content, and cricket frass on emissions of ammonia (NH_3) and carbon dioxide (CO_2) and on cricket survival. This study used a fractional factorial design with treatments repeated three times and was conducted in a temperature-controlled box (0.40 m × 0.60 m × 0.37 m). Freshly prepared and cleaned cricket frass, with adjusted moisture levels, was used. Gas production was monitored daily for 42 days. After replacing the frass with new samples corresponding to the 15 treatments, the environmental impacts on adult crickets were assessed over 7 days, during which the crickets showed a notably high survival rate. The findings indicated that temperature, moisture content, and cricket frass significantly influenced gas emissions and cricket survival rates. A higher moisture content increased the degradation of cricket frass, leading to increased microbial activity and heightened gas production. NH_3 was predominantly detected at the lower positions of the test box. Elevated levels of NH_3 (91.5 ppm) and CO_2 (1395 ppm) were observed at 40 °C temperature, 30% w.b. moisture content, and 12.86 kg/m² cricket frass. Despite environmental variations, cricket survival rates remained consistently high, ranging from 95% to 99%, particularly with low moisture content (20% w.b.) and minimal cricket frass accumulation (4.17 kg/m²). This research can assist the environmental management of low-level factors to achieve high cricket productivity. The future application for cricket farms involves managing the environment appropriately, such as cleaning cricket ponds weekly to prevent cricket frass accumulation, controlling the moisture content of food, particularly fresh plant-based food, and using watering methods that do not increase the humidity inside the cricket pond. Additionally, commercial cricket farming could be conducted in controlled temperature rooms.

Keywords: Ammonia, Carbon dioxide, Cricket frass, Cricket survival, Thailand

1. Introduction

Crickets are increasingly being recognized as a viable and sustainable diet source due to their high nutritional value and low environmental impact compared to traditional livestock [1-4]. They are highly nutritious, containing significant amounts of protein, fat, fiber, minerals, and vitamins [5-8]. Studies on a number of species have shown that crickets contain 58–78% crude protein and up to 18% fat [9-12] on a dry matter basis, as well as trace minerals [13]. Crickets have been consumed in various cultures for centuries, and interest in using crickets as part of modern diets has been growing globally. Mass rearing of insects is proposed as a solution to achieve better utilization of organic matter from the side and residual streams, aimed at producing a sufficient amount of high-quality food and feed. Several studies [14-16] have investigated the development of the edible cricket industry in Thailand. Currently, there are more than 20,000 cricket farms spread across 26 provinces in Thailand. These farms have a combined production capacity of up to 7,500 tons per year [17].

It has been observed that cricket farming aligns with the way of life, culture, and available resources in each area. The method of raising commercial crickets involves using a pond made of gypsum board, which allows for limited space requirements, easy care, and mobility. The production of cricket insects is susceptible to a range of uncertainties and is influenced by numerous factors [18, 19], such as temperature, moisture, diet feed, and the environment in the pond. Temperature is a crucial factor in insect production systems because insects are poikilothermic organisms, meaning their body temperature is dependent on the ambient temperature [20]. The developmental rate of insects depends on temperature according to an optimum curve delimited by a minimum and maximum [21, 22]. For house crickets, the optimal range is between 28 °C and 30 °C [23]. When the temperature in the rearing facility deviates from the optimal range, it can result in temperature stress, which can lead to negative physiological effects [24, 25]. Srygley [25] reported that temperature affects Mormon cricket movement, and both temperature and moisture can affect survivorship of all life stages. It was found that reproduction was optimal when the ambient temperature ranged from 30 °C to 39 °C and the insects were exposed to broad-spectrum lights. Maternal body temperature played a significant role, while large changes in moisture had very little effect on reproduction. Usually, productivity peaks during March to April, while the November to February period witnesses a decrease in output mainly because of colder weather conditions. The feeding rate and productivity tend to decrease during colder periods [26]. In temperate

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climates, the oviposition period of tettigoniids can commence as early as July or as late as October [27]. Different temperatures experienced by eggs before winter can initiate embryonic development until the final stages before hatching [28]. This study investigated the effect of summer temperature on egg diapause and the effect of winter temperature on egg survival. It was observed that temperature changes occurred during each month and corresponded to the humidity in the air. Additionally, the humidity inside the cricket pond affects crickets' food intake. Crickets can feed on a variety of foods, including agricultural by-products, vegetable materials, commercial food, forage, and even weeds [29-35]. Moreover, moisture in the surrounding pond increases with the water supply to the crickets. The effects of hydration on the growth of house crickets, *Acheta domesticus* (Linnaeus), were reported by Butnan and Duangpukdee [36].

Crickets are mass-rearing produced in Thai farming. Commercial diet feed is recommended because it is rich in nutrients such as protein, fat, fiber and moisture content of about 21%, 4%, 5%, and 13%, respectively [37]. Crickets have a high feeding rate and good productivity. However, cricket farming generates waste in the form of cricket frass and excess diet, which can accumulate in ponds. Our preliminary survey of cricket farming revealed a relationship between cricket production and frass quantity and showed that waste in a batch was 1.0–2.0 times greater than cricket production. The frass comprises excrement, body parts of dead crickets, and residues of instant feed and supplements. Mismanagement of cricket frass can potentially lead to deleterious environmental impacts, such as eutrophication and greenhouse gas emissions [38, 39]. Cricket frass is rich in plant nutrients and can likely be used as a soil fertilizer. The N, P, and K contents of the frass are 2.3–2.6%, 1.6–2.0%, and 1.8–2.3%, respectively [40, 41]. These values correspond to the findings of Butnan and Duangpukdee [36], who reported the nutrient contents in the frass of *Acheta domesticus* crickets while composting, as shown in Table 1. The decomposition of frass within the ponds may release toxic gases, such as ammonia and carbon dioxide, due to poor air circulation within the pond. The number of live crickets generally decreased with increasing exposure time, and highly concentrated gas [42] reported dietary phosphorus availability during development influences the condition and life history traits of the cricket, *Acheta domesticus* L. Therefore, the factors surrounding the pond that affected the production of crickets were the weather of seasons, temperature, and moisture in the air. An increased production of crickets leads to an increase in cricket frass. Researchers focus on factors that increase cricket production in cricket farming. However, cricket frass [36-39] is high in organic matter and is primarily used for growing plants. At the same time, the decomposition of cricket frass can impact crickets' health and diet intake [43]. Few studies have addressed the factors of cricket frass that affect the health of crickets.

Thus, this research aims to study the environmental factors of cricket ponds, namely the cricket frass, moisture content of frass, and temperature, which influence the generation of NH_3 and CO_2. These factors, in turn, impact the survival of crickets. This knowledge can improve the management of cricket farms, especially pond cleaning, and increase the productivity of cricket farms.

2. Materials and methods

2.1 Materials

The plastic pond dimensions were 0.40 m, 0.60 m, and 0.37 m in width, length, and height, respectively. These were proportional sizes compared to the gypsum board of the cricket farming, with a ratio of 1 to 10. Temperature control tools were installed using two 100-watt light bulbs positioned on the box lid to regulate temperature levels. Additionally, there were small air vents on the box lid to prevent excessive steam accumulation.

The instrument for measuring ammonia in the atmosphere was the smart sensor model AR8500 (Protronics, EXTECH Instruments, Thailand), which had a measurement range of 0.0–99.9 ppm with an accuracy of ± 2%. The carbon dioxide measuring instrument was an EXTECH CO_2 gas meter (Protronics, EXTECH Instruments, Thailand), which was capable of measurement in the range of 0–9,999 ppm.

Table 1 Chemical and physical properties of cricket frass.

Chemical property	**(lb/ton)[44]**		**(%)[36]**
Total N	97.1	N	4.38
NH_3	4.3	P	2.97
Organic N	92.8	K	2.37
P_2O_5	60.8	CaO	15.53
K_2O	28.2	MgO	5.87
Ca	51.6	S	9.80
Mg	6.6	Organic carbon	40.20
Na	7.2	Organic matter	69.30
Cu	0.04		
Zn	0.38		
Fe	1.28		
Mn	0.22		
Physical property*			
Bulk density** (g/cm^3)	0.401		
Specific gravity (-)	1.670		
D10 (mm)	0.273		
D50 (mm)	0.927		
D90 (mm)	1.486		
Fineness modulus	1.248		

*Owner test; **Loosely packed in the cylinders of 500 cm^3 volume, 10 cm diameter in five replicates

2.2 Sample preparation

Preparation of cricket frass samples in experiments recording cricket survival, NH_3, and CO_2 production involved collecting waste material from cricket farmers in Honghee Village (N16^o 22' 53.4714", E103^o 18' 4.464"), Yang Talat District, Kalasin Province, Thailand immediately after the harvest. Table 1 shows the chemical and physical properties of cricket frass, highlighting its high fertilization potential, especially with a total nitrogen content of 97.1 lb/ton and an organic matter content of 69.30%. Furthermore, the sieve analysis indicates a median grain passing D50 of 0.927 mm and a fineness modulus of 1.25. The cricket frass sample was cleaned by removing plant residues and the carcasses of dead crickets. Then, samples were randomly selected to determine the moisture content by drying the samples 105 ℃ for 72 hours and calculating the moisture percentage [45]. The obtained moisture content served as the initial value, and water was added as needed to achieve the desired sample moisture level.

2.3 Factors and factor levels

The determination of factors and their levels was based on findings from cricket farming, in which it is known that production variability depends on seasons or weather conditions [18, 19, 39]. The season is strongly related to temperature and moisture. The Meteorological Department Station in Maha Sarakham Province, Thailand, reported that annual temperatures ranged from 22 ℃ to 40 ℃ in 2023. However, this experiment was conducted from November to December, so the minimum temperature was set according to environmental conditions at around 28 ℃. The moisture factor of cricket frass samples was randomly selected from various farms that used different cricket farming feeding approaches. The sample revealed a low moisture content of 20% wet basis (w.b.) in farms commercial feeding crickets and a high moisture content of 30%w.b. in farms that commercial feeding crickets with prepared supplemented with vegetables, with a high incidence of cricket mortality or carcasses. Meanwhile, the cricket frass factor in the gypsum board cricket pond ranged from 12 kg to 36 kg depending on the yield and feed used for cricket farming. When calculated per area, it ranged from 4.17 kg/m^2 to 12.86 kg/m^2. Therefore, based on the factors of temperature, moisture, and cricket frass, an additional middle-level factor was established as a factor of temperature (28 ℃, 35 ℃, and 40 ℃), moisture (20%w.b., 25%w.b., and 30%w.b.), and cricket frass (4.17 kg/m^2, 8.68 kg/m^2, and 12.86 kg/m^2), with lower, middle, and upper levels of the factors [46]. These factors and levels were designed using a fractional factorial design with 15 treatments, and each treatment was repeated three times, as detailed in Table 2. These factors were grouped into a low-level factor group comprising treatments 1, 4, 6, and 11 and a high-level factor group comprising treatments 5, 10, 12, and 15. The remaining treatments formed the moderate-level factor group.

Table 2 Fractional factorial design for the experiment.

Treatment number	Temperature (℃)	Moisture content (%w.b.)	Cricket frass (kg/m^2)	Response
1	28	20	4.17	
2	28	25	8.68	
3	28	25	12.86	
4	28	30	4.17	
5	28	30	12.86	
6	35	20	4.17	
7	35	20	12.86	
8	35	25	8.68	
9	35	30	4.17	
10	35	30	12.86	
11	40	20	4.17	
12	40	20	12.86	
13	40	25	4.17	
14	40	25	8.68	
15	40	30	12.86	

Note: The low-level factor group consisted of treatments 1, 4, 6, and 11. The high-level factor group consisted of treatments 5, 10, 12, and 15. The moderate-level factor group consisted of treatments 2, 3, 7, 8, 9, 13, and 14.

2.4 NH_3 and CO_2 emissions

Cricket frass was prepared to adjust the moisture content as needed and was then put into plastic boxes. Each box contained 1.0 kg, 2.1 kg, and 3.1 kg, corresponding to the cricket frass ranges of 4.17 kg/m^2, 8.68 kg/m^2, and 12.86 kg/m^2, respectively. The lids were closed, and the temperature was controlled according to each condition's requirements. Before the gas measurement, the lid of the sample box is left open for approximately 10 minutes to allow gas dispersion and ensure a lower reduction value, consistent with the open-air cricket pond in a farm setting for convenient air exchange. The gas quantity depended on the position and time of measurement. Therefore, preliminary testing of treatments 1, 8, and 15 (low, middle, and high levels) was conducted for 7 days, with gas measurements taken once a day at around 14:00, the time of the highest temperature. Measurements were taken at nine locations: left, center, right (horizontally), bottom, middle, and top (vertically within the box). The gas data were determined for specific gas positions under all treatment conditions throughout the 42-day experiment.

2.5 Environmental factors in ponds affecting cricket survival

In addition to studying the impact of temperature, moisture content, and cricket frass in the rearing pond, which also affect NH_3 and CO_2 emissions, we examined how these factors influenced cricket survival. To ensure the pond environment was conducive to raising crickets, we conducted a 7-day survival test with 120 adult *Acheta domesticus* crickets, comprising 60 males and 60 females. These crickets were subjected to 15 treatments with the same conditions, as shown in Table 2. During the test, they were provided with a sufficient amount of commercial diet for daily consumption, with any leftover diet promptly removed from trays to prevent waste accumulation in the experimental pond. Data from this test were used to estimate the cricket survival.

2.6 Data analysis

The factors of temperature, moisture content, and cricket frass that affect the survival of crickets, NH_3, and CO_2 emissions were analyzed to assess their main and interactive effects. The results obtained were subjected to an analysis of variance and comparison of means using Tukey's Honestly Significant Difference (HSD) test with IBM SPSS Statistics 29 software (Released 2023. IBM SPSS Statistics for Windows, Version 29.0.2.0 Armonk, NY: IBM Corp). Additionally, the survival rate and NH_3 and CO_2 emissions for each week are presented, and the relationship between these indicators is examined.

3. Results and discussion

3.1 Preliminary testing for NH_3 and CO_2 emission positions

Figure 1 illustrates the NH_3 and CO_2 concentrations at low (treatment 1), moderate (treatment 8), and high (treatment 15) factor levels across various measurement points within the test pond. Notably, NH_3 (Figure 1a) exhibited the highest detectability at the bottom measurement positions. As the positions moved toward the middle and upper levels, the values decreased. NH_3 concentrations at the left, middle, and right positions (Figure 1c) were measurable in treatments 1, 8, and 15. CO_2 (Figure 1b and d) was detectable at all measurement positions and under all conditions, indicating its widespread distribution throughout the test pond area. This is attributed to its abundance in the atmosphere. Hence, opting for measurements at the bottom positions of the test box, particularly at the left, middle, and right positions, captured both NH_3 and CO_2 gases during the 42-day testing period, aligning with the lifecycle of cricket farming practices conducted by farmers.

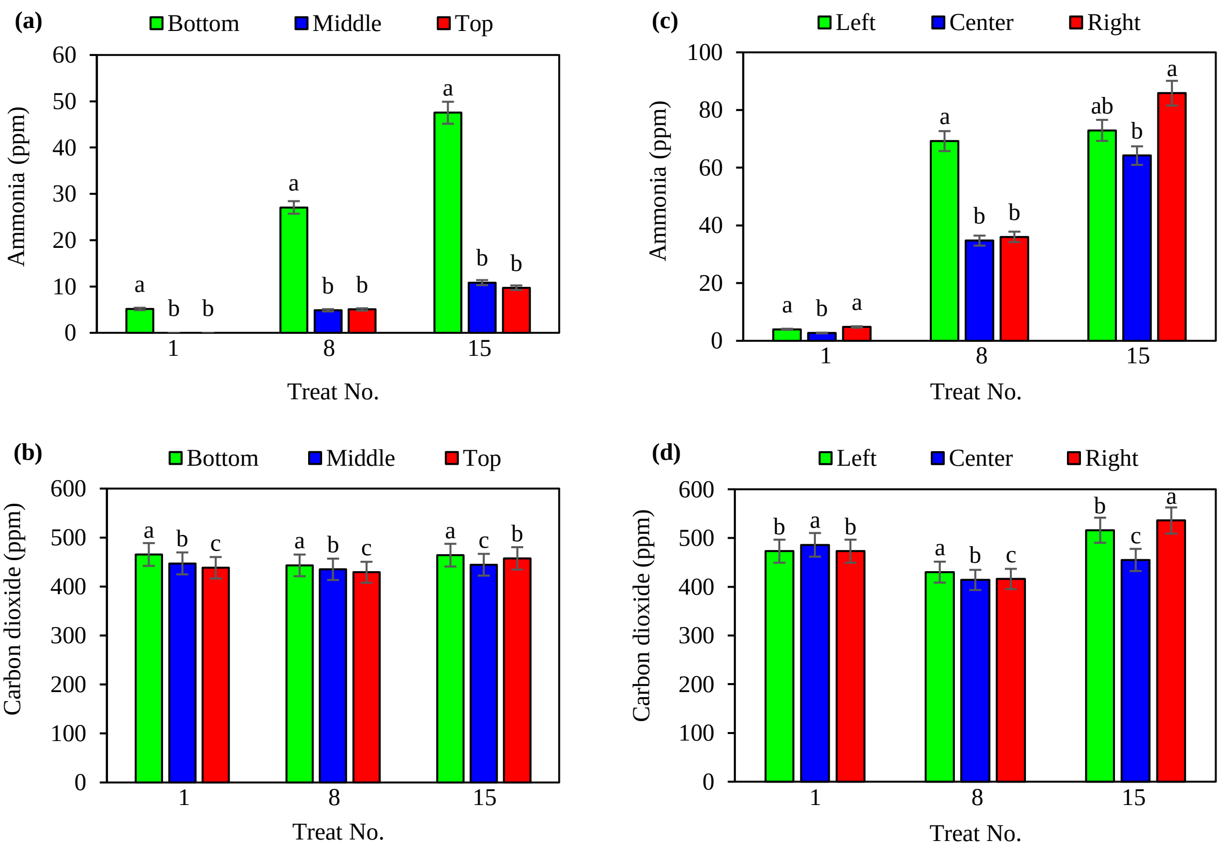

Figure 1 Preliminary testing for NH_3 and CO_2 emission positions, low-level (treatment 1), moderate-level (treatment 8), and high-level (treatment 15) factors. (a) and (b) measured at the bottom, middle, and top positions, and (c) and (d) measured at the left, center, and right positions.

3.2 Factors affection to NH_3 and CO_2

Table 3 displays the statistical analysis of NH_3 and CO_2, emphasizing the primary factors of temperature, humidity, and cricket frass, along with their interactions. The analysis was conducted using data from a week to calculate average values, acknowledging the fluctuating nature of these indicators over time. Consequently, only data from the initial week, which exhibited the highest rates, were included. The results indicate statistically significant effects for all main factors ($p < 0.0001$). However, NH_3 showed no significant impact on the interaction between humidity and cricket frass. This underscores the significant effects of the studied factors and their levels on NH_3 and CO_2 with a high level of confidence ($R^2 = 0.999$).

3.3 NH_3 and CO_2 behavior

Cricket feed is a staple diet containing up to 21% protein, which stimulates growth [47]. Crickets have short intestines, so when they consume high-protein diets, it is not fully digested and is excreted as waste in the form of uric acid. This uric acid can be enzymatically digested by uricase and converted to urea, producing gaseous nitrogen and ammonia, with oxygen acting as a catalyst for the reaction. Uric acid is converted to urea, resulting in the production of ammonia and carbon dioxide [48, 49].

Figure 2a and Figure 2b depict the patterns of NH_3 and CO_2 production, respectively. Both gases exhibited rapid increases followed by gradual decreases over 42 days. The high-level factor group had higher gas emissions than the moderated and low-level factor groups. Cricket feed, which contains a high amount of protein, resulted in the excretion of uric acid, urea, and significant nitrogen content, as reported by Olesen and Sommer [50]. Exposure to elevated temperatures led to the hydrolysis of urea and uric acid, resulting in rapid NH_3 release into the atmosphere, peaking between days 2–7 at levels of 80–100 ppm, consistent with reports indicating that NH_3 is generated rapidly from the action of bacteria on organic nitrogen substances, accounting for up to 35% of the entire process within the first 2 to 3 days [51-53]. The peak production of CO_2 (Figure 2b) also exhibited a rapid increase from 1000 ppm to 1744 ppm over 1 to 3 days, followed by a sharp decline, indicating that this gas had a weight close to that of air, as it is a component of normal air at 400–500 ppm [54]. Therefore, these conditions facilitated easy dispersion into the atmosphere under normal conditions.

Table 3 Analysis of variance for the fractional factorial design.

	R^2	*df*	*F* value	*P* value
Ammonia	0.999			
Temperature (°C)		2	714.363	<0.0001
Moisture content (%)		2	18415.976	<0.0001
Cricket frass (kg/m^2)		2	775.433	<0.0001
Temperature × moisture content		2	26.673	<0.0001
Temperature × cricket frass		2	36.518	<0.0001
Moisture content × cricket frass		2	0.370	0.548
Carbon dioxide	1.000			
Temperature (°C)		2	3022.113	<0.0001
Moisture content (%)		2	23759.414	<0.0001
Cricket frass (kg/m^2)		2	28892.047	<0.0001
Temperature × moisture content		2	488.408	<0.0001
Temperature × cricket frass		2	254.633	<0.0001
Moisture content × cricket frass			2173.244	<0.0001

Note: Significantly different levels ($p < 0.05$) were determined according to the HSD range test with degrees of freedom (df) = 2.

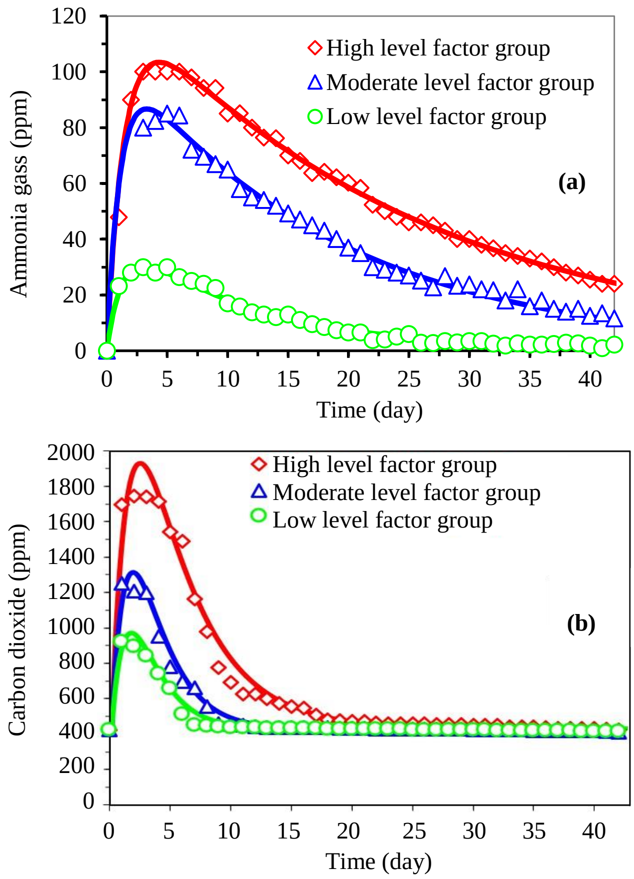

Figure 2 (a) NH_3 and (b) CO_2 emission over 42 days.

Figure 3 illustrates NH_3 and CO_2 levels in the low-level factor group, moderate-level factor group, and high-level factor group. This treatment condition corresponded with NH_3 values ranging between 22.37 ppm and 27.54 ppm, 36.08 ppm and 67.41 ppm, and 78.10 ppm and 94.58 ppm, respectively. CO_2 values ranged between 400 ppm and 500 ppm, 626.09 ppm and 944.42 ppm, and 1298.10 ppm and 1594.04 ppm, respectively. Conditions with elevated levels of these factors promote microbial growth and the breakdown of organic materials, leading to increased gas release. Therefore, variations in temperature, humidity, and the cricket frass will significantly impact the production of NH_3 and CO_2 emissions.

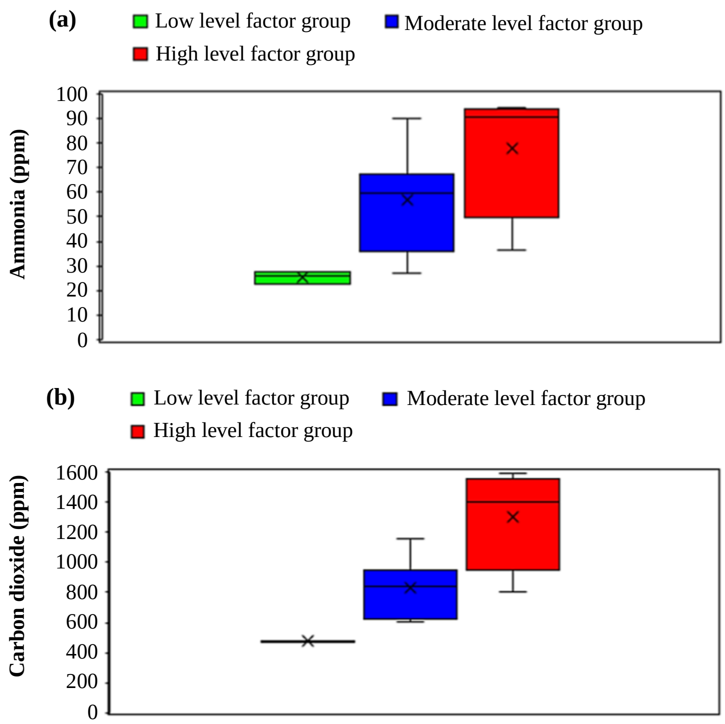

Figure 3 The level factor treatment group affected by (a) NH_3 and (b) CO_2.

Figure 4 illustrates the interactions of NH_3 and CO_2 with temperature (Figure 4a), humidity (Figure 4b), and cricket frass (Figure 4c). Discrepancies are indicated, and letters denote statistical differences. With increasing temperature, NH_3 and CO_2 values rose at similar rates, exhibiting statistically significant differences across the temperature range of 28–40°C. Conversely, humidity significantly increased NH_3 and CO_2 values across the humidity range, with NH_3 showing a faster rate of increase than CO_2. NH_3 had a slope of 7.80, and CO_2 had a slope of 6.44. Both NH_3 and CO_2 increased at similar rates with increasing amounts of cricket excrement. However, at moderate to high levels of cricket frass, CO_2 increased at a significantly higher rate than NH_3. Additionally, according to reports by [55], NH_3 formation depends on nitrogen concentration, pH, temperature, wind speed, and microbial activity. Large emission areas and high wind speeds may increase NH_3 evaporation. It was demonstrated that NH_3 increased nonlinearly with temperature, air velocity, and solution pH and linearly with total nitrogen concentration. Environmental temperature control can decrease the production of ammonia gas and carbon dioxide, which results from the breakdown of organic matter. This is due to cricket frass acting as a food and habitat source for microorganisms, thereby reducing microbial growth and the breakdown of organic material.

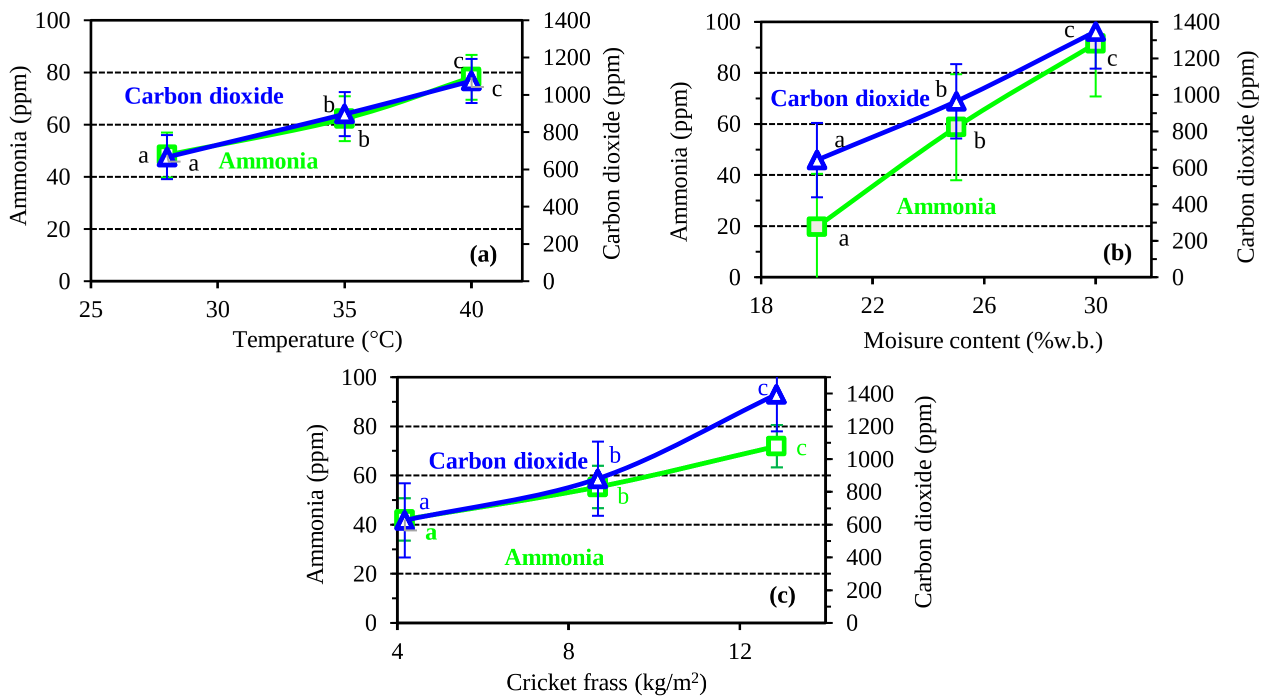

Figure 4 Interaction between NH_3 and CO_2 with (a) temperature, (b) moisture content, and (c) cricket frass.

3.4 Survival

To ensure the pond environment is conducive to raising crickets, we conducted a 7-day survival test with a total of 120 adult crickets, comprising 60 males and 60 females. These crickets were subjected to 15 different treatment conditions. During the test, they were provided with a sufficient amount of commercial diet for daily consumption, and any leftover diet was promptly removed from trays to prevent waste accumulation in the experiment. Figure 5 shows the survival of cricket versus treatments, and Figure 6 shows the mortality of cricket NH_3 and CO_2 related to the level factor treatment group.

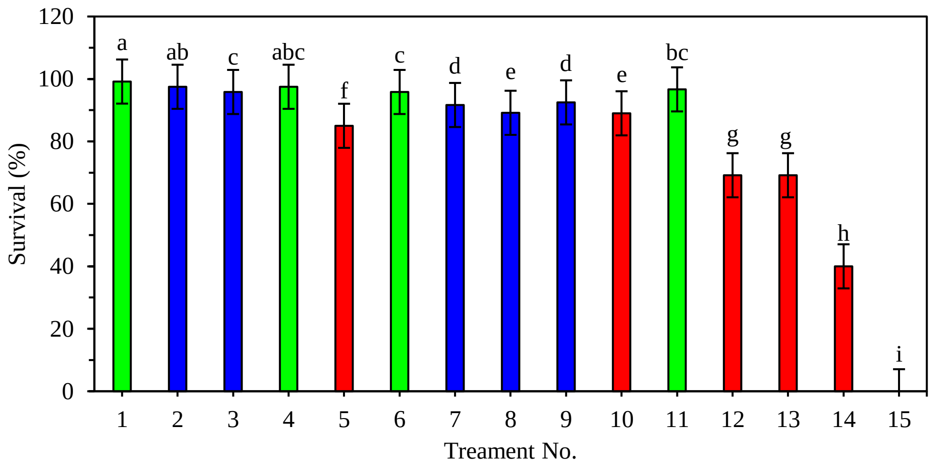

Figure 5 The survival of crickets compared to the treatments.

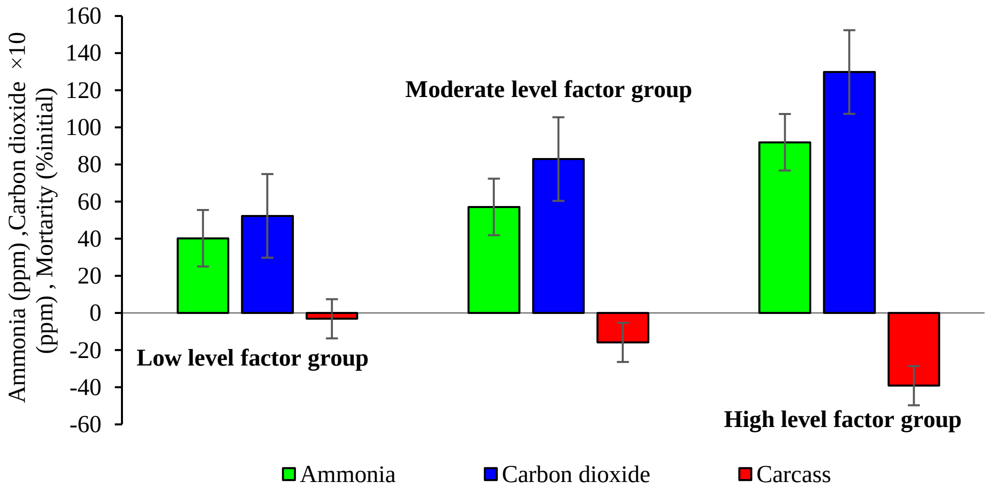

Figure 6 The mortality, NH_3, and CO_2 related to level factor treatment group.

The test results revealed a high average survival rate of crickets, ranging from 95% to 99% when the moisture content and cricket frass in the pond were low, at 20%w.b. and cricket frass 4.17 kg/m^2 (treatments 1, 2, 3, 4, 6, and 11). This indicates a correlation with the emissions of NH_3 and CO_2 in the pond, as depicted in Figure 6. The low-level factor group corresponded to low NH_3 (40.26 ppm) and CO_2 (520.30 ppm) production, with cricket mortality at only 3.12%. This finding suggests there is insufficient growth of microorganisms, resulting in low gas production and, consequently, low cricket mortality. Conversely, NH_3 and CO_2 levels were high in the moderate-level factor group and high-level factor group, with NH_3 values at 57.12 ppm and 91.97 ppm and CO_2 values at 820.91 ppm and 1290.97 ppm, respectively, accompanied by cricket mortality rates of 15.83% and 36.87%, respectively. Therefore, lower levels of temperature, humidity, and cricket frass lead to reduced NH_3 and CO_2 emissions and higher cricket survival rates, making it suitable for use in the management of cricket farms.

4. Conclusions

The results showed that temperature, moisture content, and cricket frass significantly affected the emission of gas and survival. The increased moisture content will lead to higher degradation of cricket frass, as microorganisms will grow and multiply, resulting in increased gas production. The NH_3 was found at the bottom positions of the test pond, specifically at the left, middle, and right positions. The factors affecting high NH_3 and CO_2 were 91.5 ppm and 1395 ppm, respectively, at a temperature of 40 °C, moisture content of 30 %w.b., and cricket frass of 12.86 kg/m^2. Furthermore, if the cricket frass increases due to the accumulation of cricket feed, NH_3 and CO_2 levels will continuously increase significantly. The survival rate of crickets was high, ranging from 95% to 99% when the moisture content and cricket frass in the pond were low, at 20%w.b. and mostly 4.17 kg/m^2. The findings of this study could be useful for farmers and researchers in developing sustainable and efficient management for cricket production.

5. Acknowledgements

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