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Respiration of *Tribolium castaneum* (Herbst) at Different Oxygen Concentrations

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Abstract: The main content of this research is the respiration rate of the egg, larvae of 7 – 10d instars (young larvae), larvae of 18 – 22d instars (old larvae), pupae and adults of Tribolium castaneum (Herbst) exposed to atmospheres containing 21%, 15%, 10%, 5%, 3%, 2% and 1% oxygen in nitrogen at 30℃. The respiration rate (RR) of them was determined with Warburg respiration instrument. The results indicated that respiration rate of the five stages was different at different oxygen atmospheres. At 21% and 15% oxygen atmospheres, RR of egg was the least, followed by young larvae, adult, pupae and old larvae. At 10% and 5% oxygen atmospheres, RR of young larvae was the least followed by egg, adults, pupae and old larvae. At 3%, 2% and 1% oxygen atmospheres, RR of young larvae was similar to egg, followed by adults, pupae and old larvae. Obviously, the downtrend of RR of the five stages was different, RR of old larvae was the highest, RR of egg and young larvae was the least, and the value of them was at the same level. With the reduction of, the model of RR at the different stages there was a decrease of the logarithm, which is $y(egg) = 0.44\ln(x) + 0.12$, $y(young\ larvae) = 0.52\ln(x) + 0.01$, $y(old\ larvae) = 0.90\ln(x) + 1.22$, $y(pupae) = 0.63\ln(x) + 0.57$ and $y(adult) = 0.36\ln(x) + 1.08$. The decreasing rate of the RR of the five stages was different, the reduction in the rate of old larvae was the greatest, followed by pupae, young larvae and egg and the last was the adult. At reduced oxygen levels, the decrease of respiration rates of all stages was proportional to the oxygen levels. Under 10% oxygen atmospheres, RR of two stages of larvae were significantly different ($a = 0.01$). Under 5% oxygen atmospheres, RR of egg and adult were significantly different ($a = 0.01$). And under 3% oxygen atmospheres, RR of pupae was markedly restrained and differed significantly ($a = 0.01$). Hence, the reduction of RR and the reduction in degree of RR were related to the death of the insects in low oxygen atmosphere.

Key words: respiration rate, *Tribolium castaneum*, low oxygen, stages

Preface

At present, although chemical fumigants are still extensively used as major pesticides for stored products in various fields, the substantial use of chemical fumigants can result in certain problems such as residues on food, environmental pollution and etc., Countries have already begun to limit the use of chemical pesticides and and qre developing registration of new chemical pesticides gradually. In consideration that the status of methyl bromide will be eliminated gradually in the whole world, the high PH_3 resistance of pests and some new-type fumigants can be used for only on special products and situations, and the developments of the new-type fumigants which are green, environmental-friendly and effective are necessary. Low oxygen used as a gas pest killing technology has common advantages over fumigants. It is a grain storage technology which can kill the pests or inhibit the growths and developments of pests through reducing the oxygen content in the environment by natural or artificial methods. One can also can raise the temperature and increase the CO_2 content in the environment to achieve a more effective and more rapid controlling effect. It is obvious that low oxygen grain storage technology has advantages such as safety, green and has no environmental pollution. It conforms to the green grain storage idea which is promoted strongly in China.

At present, CO_2 rich and low oxygen grain storage technology is popularized in China. We need to understand the respiration characteristics of stored grain pests under the low oxygen condition to explain the mechanism of pest control at low oxygen for the improvement of modified atmospheres technology. We need to provide the theoretical basis for application of the low oxygen pest controlling technology. The purposes of this experiment were 1) to research the

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respiration changes of *Tribolium castaneum* (Herbst) under the low oxygen condition through determination of its respiration rates at various stages under different oxygen concentrations, 2) provide a basis for research on mechanism of pest killing by low oxygen and 3) recommendation of the effective concentration for controlling of stored grain pests by low oxygen. At the same time, the research on respiration rates of insects under low oxygen condition has significant meaning for grain temperature rises caused by heat production of the insect's metabolism and physiological reactions of insects to different environments[1].

Materials and Method

Materials

Instruments and equipments

SKW-3 type micro-respiration apparatus, Shanghai Gongda Industry and Trade Corporation;

Electric thermostatic incubator, Shanghai Precision Instruments Co., Ltd.

Orsat gas analyzer, Shangdong Leling Xinghua Glass Instruments Factory

Reagents

Industrial oxygen, purity ≥ 99. 7%; high purity nitrogen, purity ≥ 99. 9996%. All produced by Beijing Bei Temperature Gas Factory.

Test insect

The test insect was *T. castaneum* which had been cultured in the Academy of State Administration of Grain for dozens of generations, and was taken from the Yiyang depot, State Grain Reserves.

Test Method

Cultivation of the test insects

Clean the wheat and place it into an oven of 80℃. adjust the water content, then store in refrigerator for use. Prepare the feed with whole wheat flour and yeast at the ratio of 95:5 for culture of *T. castaneum*. Add *T. castaneum* into the prepared feed and culture it under the condition of temperature being 30 ± 1℃ and relative humidity (RH) being 75% ± 5%. Record the kind, species and culture date of the insect. After 3 days, screen the adults out and put them into another prepared culture bottle to culture them, and put the screen underflow which contain the eggs back into the original bottle and culture them, and perform tests for the test insects at various stages and ages [2].

Selection of the test insects

Confirmed days of the test insects by stages and ages and the necessary quantities of the test insects for each treated group are shown in table 1.

Table 1. Stages and quantities of the test insects

Stages	Quantity(/group)	Age(d)
egg	400	0 – 1
Young larva	50	7 – 10
Old larva	10	18 – 22
Pupa	10	1 – 2
Adult	10	10 – 14

Note: the age of larva is calculated from egg, the age of pupa is calculated from pupation and the age of adult is calculated from eclosion.

Test method

Use SKW-3 type micro-respiration apparatus, according the standard pressure reduction procedure[3], at 30℃, perform test of respiration rates for the test insects under the conditions of which oxygen concentrations are 21%, 15%, 10%, 5%, 3%, 2% and 1% separately.

Test method of respiration rate under the normal oxygen concentration

Perform test of oxygen consumption (per hour) for test insects under the condition of temperature being 30℃ and oxygen concentration being 21%. Perform parallel test for 4 groups.

Test method of respiration rate under low oxygen condition

According to the characteristic of piezometric pipe of SKW-3 type micro-respiration apparatus, set the upper mouth of the piezometric pipe as the air inlet, and the mouth at the side of the reaction bottle as the air outlet. Charge the mixed gas of nitrogen and oxygen at a certain ratio from the upper mouth of the piezometric pipe into the reaction bottle and open the side mouth of the reaction bottle at the same time to discharge the original gas in the reaction bottle, and thus create the needed low oxygen environment through this gas discharging method. Perform the test of gas concentration with an Orsat gas analyzer. When the gas concentration in the reaction bottle reaches the requirement of the test, stop venting and test the airtightness after sealing. Determine if the airtightness is good or bad through observation and see if there is difference in height between two liquid levels in the U-tube of the piezometric pipe.

At 30℃, perform the test of oxygen consumption (per hour) for the test insects under the conditions of oxygen concentrations being 15%, 10%, 5%, 3%, 2% and 1%. Separately, perform parallel test for 4 groups.

Weighing

Take out the test insects after above test finished and dry them at 80℃ to constant weight, and record the data.

Data processing method

According to calculation formula of respiration rate: respiration rate = oxygen consumption/(weight of test insect × time), perform calculation on collected original data to obtain the oxygen consumption each hour and per mg of test insect, i. e. respiration rate.

Analyze the change trend of oxygen consumption in unit time for the test insect under different oxygen concentrations with EXCEL software; perform analysis of variance on oxygen consumption in unit time for *T. castaneum*at various stages under seven different oxygen concentrations described above with SAS (Statistics Analysis System) data processing software and compare significance of effects of different low oxygen concentrations on respiration rate.

Results and Analysis

Respiration Rates of the Egg of *T. castaneum* under Different Oxygen Concentrations

From Fig 1, we can see that under the conditions of oxygen concentrations being 21%, 15%, 10%, 5%, 3%, 2% and 1%, the corresponding respiration rates of the egg of *T. castaneum* are 1.50 ± 0.15, 1.43 ± 0.13, 1.11 ± 0.19, 0.78 ± 0.06, 0.36 ± 0.04, 0.38 ± 0.04 and 0.32 ± 0.04 (μl/mg · h). Analytically, there is a logarithmic relationship between respiration rate of the egg of *T. castaneum* and oxygen concentration, and the mathematical model is $y = 0.44\ln(x) + 0.12$ ($R^2 = 0.92$). From the analysis of variance of the data, we see that during the process of oxygen concentration reduction from 21% to 1%, the respiration rate of egg reduces gradually. When the oxygen concentration is reduced to 5%, the respiration rate of the egg is strongly reduced. (a = 0.01). This indicates that when the oxygen concentration is below 5%, the respiration rate of egg is inhibited strongly by the low oxygen environment.

Respiration Rates of the Young Larva of *T. castaneum* under Different Oxygen Concentrations

From Fig 2, we can see that under seven oxygen concentrations from 21% to 1%, the corresponding respiration rates of the young larva of *T castaneum* are 1.75 ± 0.07, 1.64 ± 0.13, 0.96 ± 0.06, 0.55 ± 0.01, 0.45 ± 0.01, 0.38 ± 0.05 and 0.25 ± 0.03 (μL/mg · h.)

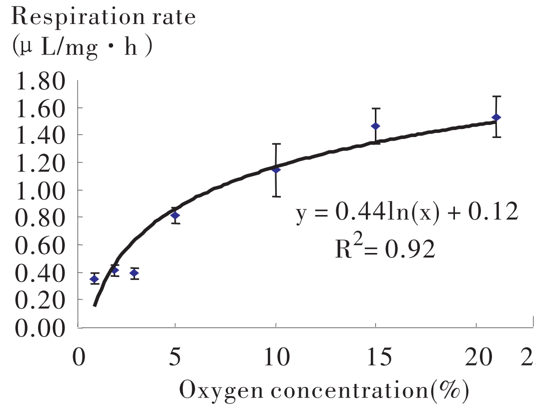

Fig. 1 Respiration rate of the egg of *T. casta* neumunder different oxygen concentrations at 30℃

Analytically, there is a logarithmic relationship between respiration rate of the young larva of *T. casta*neum and oxygen concentration, and the mathematical model is $y = 0.52\ln(x) + 0.01$ ($R^2 = 0.87$). The model type is the same as that of the egg, except that of the coefficient. From analysis of variance for the data, we can see that during the process of oxygen concentration reduction from 21% to 1%, the respiration rate of the young larva of *T. casta*neum reduces gradually. When the oxygen concentration is reduced to 10%, the respiration rate of the young larva reduces greatly (a = 0.01). This indicates that when the oxygen concentration is below 10%, the respiration rate of the young larva is inhibited strongly by the low oxygen environment

Comparison of Respiration Rates of the old Larva of *T. castaneum* under Different Oxygen Concentrations

From Fig 3, we can see that at 30℃ and the same oxygen concentration series, the corresponding respiration rates of the old larva of *T. castaneum* are 3.99 ± 0.19, 3.60 ± 0.06, 3.22 ± 0.06, 2.74 ± 0.06, 2.11 ± 0.13, 2.02 ± 0.05 and 1.13 ± 0.03μL/mg · h. Analytically, there is a logarithmic relationship between respiration rate of the old larva of *T. castaneum* and oxygen concentration, and the mathematical model is $y = 0.90\ln(x) + 1.22$ ($R^2 = 0.99$). The model type is the same as that of the egg and young larva, except for the coefficient. From the analysis of variance data, we see that with the reduction of oxygen concentration, the respiration rate of the old larva is reduced gradually; when the oxygen concentration reduced to 10%, the respiration rate of the old larva is greatly reduced (a = 0.01) This indicates that when the oxygen concentration is below 10%, the respiration rate of the old larva is strongly inhibited by the low oxygen environment.

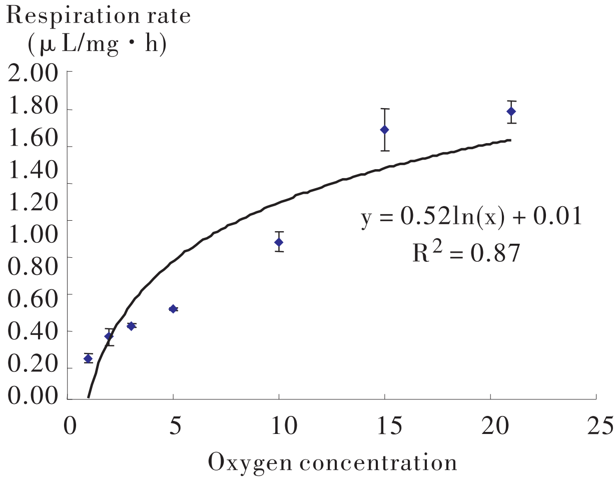

Fig. 2 Respiration rate of the larva (7 – 10 days) of *T. castaneum under* different oxygen concentrations at 30℃

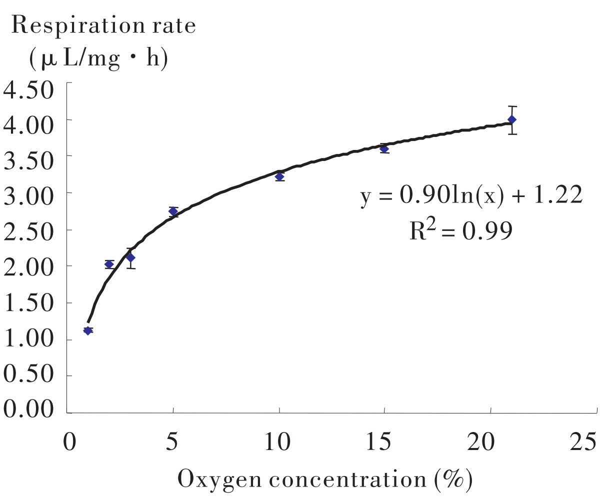

Fig. 3 Respiration rate of the larva (18 – 22 days) of *T. castaneum* (Herbst) under different oxygen concentration

Comparison of Respiration Rates of the Pupa of *T. castaneum* under Different Oxygen Concentrations

From Fig 4, we can see that under the same test condition described above, the corresponding respiration rates of the pupa of *T. castaneum* are 2. 34 0. 04, 2. 17 ± 0. 15, 2. 08 ± 0. 17, 1. 98 ± 0. 01, 1. 53 ± 0. 03, 0. 69 ± 0. 02 and 0. 47 ± 0. 01 μL/mg · h. Analytically, at 30℃, there is a logarithmic relationship between respiration rate of the pupa of *T. castaneum* and oxygen concentration, and the mathematical model is $y = 0.63\ln(x) + 0.57$ (R^2 = 0. 89). The model type is the same as those of those three test insects described above. From analysis of variance of the data, we see that with the reduction of oxygen concentration, the respiration rate of the pupa is gradually reduced; but the difference with the above three test insects is that when the oxygen concentration is reduced to 3%, the respiration rate of the pupa is greatly reduced (a = 0. 01). This indicates that the respiration rate of the pupa is strongly inhibited by the low oxygen environment.

Comparison of Respiration Rates of the Adult of *T. castaneum* under Different Oxygen Concentrations

From Fig 5, we can see that under the same test condition described above, the respiration rates of the adult of *T. castaneum* are correspondingly 2. 05 ± 0. 03, 1. 97 ± 0. 04, 1. 95 ± 0. 08, 1. 80 ± 0. 04, 1. 61 ± 0. 03, 1. 49 ± 0. 05 and 0. 79 ± 0. 02 μL/mg · h. Analytically, at 30℃, there is a logarithmic relationship between respiration rate of the adult of *T. castaneum* and oxygen concentration, and the mathematical model is $y = 0.36\ln(x) + 1.08$ (R^2 = 0. 85). From analysis of variance of the data, we see that during the process of oxygen concentration reduction from 21% to 1%, the respiration rate of the adult reduces gradually. When the oxygen concentration reduces to 5%, the respiration rate of the adult reduces greatly (a = 0. 01). This indicates the respiration rate of the adult is inhibited strongly by the low oxygen environment and this result is the same as that of the egg.

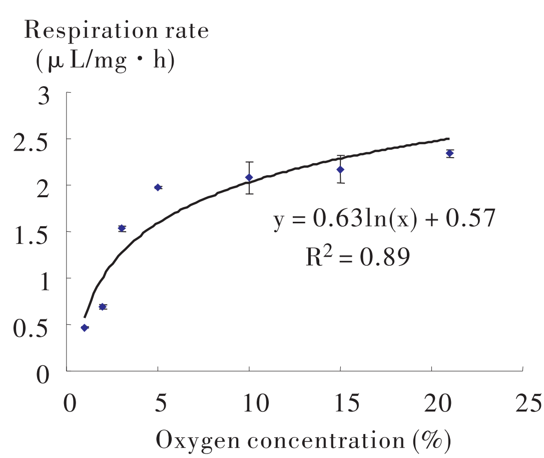

Fig. 4 Respiration rate of the pupa of *T. casta – neum* under different oxygen concentrations at 30℃

Discussion

Shijun performed research on respiration rates of the pupa of *Cacoecimorpha pronubana* under oxygen concentrations of 21%, 10%, 8%, 6%, 4%, 2% and1% [4]. Emekci reported on respiration rates of eggs, young larvae, old larvae, pupae and adults of *T. castaneum* and *Rhizopertha dominica* (Fabricius) under oxygen concentrations of 21%, 15%, 10%, 5%, 3%,

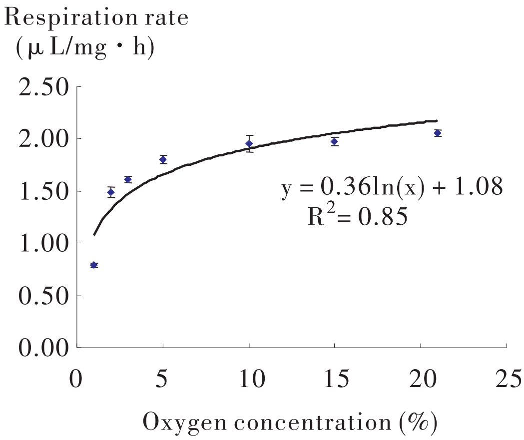

Fig. 5 Respiration rate of the adult of *T. castaneum* under different oxygen concentrations at 30℃

2% and 1%[5,6]. They found that with reduction of the oxygen concentration, the corresponding respiration rates of the test insects were gradually reduced, which is in agreement with the findings of the present article.

Navarro found that when the oxygen concentration was reduced to 3%, the respiration rate of *Ephestia cautella* (Walker) was strongly inhibited [6]. White (1981) and Campbell (1990) reported on respiration rates of *Oryzaephilus surinamensis* Linne and *Cryptolestes ferrugineus* (Stephens) and obtained the same results[7,8]. In the present research it was also found that, when the oxygen concentration was reduced to 3%, the respiration rates of *T. castaneum* at various stages were all strongly inhibited.

For most insects, gas exchange is performed through a tracheal system. According to the difference in the pressure gradient of oxygen, oxygen will enter the tissue of insect from a step-wised branched trachea, through the spiracles. When oxygen exists, energy producing substances will be oxidized and decomposed to CO_2 and H_2O and will produce considerable ATP. However, under a low oxygen environment, the partial pressure of oxygen in air is relatively small, which inhibits the input of oxygen to some extent and results in decreasing of respiration rate of insect and reduction of energy supply. Since the energy supply is reduced the insect will increase ATP production through anaerobic respiration glycolysis. But it still can not supply the normal energy for the insect survival[9]. A product of anaerobic respiration-lactic acid has a harmful effect on insect[10]. It is obvious that under low oxygen condition, the reduction of the respiration rate of insect can result in a deficiency of APT production and accumulation of toxic metabolites which finally leads to the death of insect. The findings of the present work show that the effects of the low oxygen on respiration rates of *T. castaneum* are different at various stages of the development of the insect. When the oxygen concentration is ≤ 10%, the reductions of respiration rates of the young larva and old larva of *T. castaneum* are significant. When the oxygen concentration is ≤5%, the reduction of respiration rate of the egg and the adult of *T. castaneum* is significant. When the oxygen concentration is ≤3%, the reduction of respiration rate of the pupa of *T. castaneum* is significant. However, when the environmental oxygen concentration is below 3%, the respiration rates of the egg, larva, pupa and adult of *T. castaneum* are significantly inhibited. Current researche shows that when the oxygen concentration is below 3%, each stage of the store grain pest can be controlled effectively[11]. Annis reported that the low oxygen concentrations of 1% –5% has lethal effect on the adults of stored grain pests[12]. The present article shows that when the oxygen concentration is 1% – 5%, the respiration rate of the adult of *T. castaneum* is obviously inhibited. The reduction of the respiration rates of stored grain pests under the low oxygen condition can represent differences in sensitivities of pests to the low oxygen environment, and the mathematical models developed on respiration rates of stored grain pests under the low oxygen condition help us establish more economic and more effective stored grain pests controlling strategy.

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