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The Inhibition Effect of Low Oxygen on Four Species of Stored Grain Insect Pests

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Abstract: Four kinds of representative stored grain pests; *Tribolium castaneum* Herbst, *Oryzaephilus surinamensis Linne*, *Sitophilus zeamais* Motschulsky and *Sitotroga cerealella* Olivier, were placed into the low oxygen environments attemperature of 30℃. The oxygen contents were 5%, 10% and 15%. Rresearch was performed the on the life history, growth and development and the inhibition effects under the different low oxygen environments…The results showed that when compared with the same kinds of test pests grown and developed under oxygen content of 21%, the eggs of these kinds of test pests could not be incubated under a low oxygen environment of 5%, and they could not finish their life history. A low oxygen environment of 10% can inhibited the incubation of the eggs of *T. castaneum*, *O. surinamensis* and *S. cerealella* obviously, and it also had a certain inhibition on the developments of larvae of *T. castaneum* and *O. surinamensi* s This results in prolongation of the larva period. . The larva of *S. cerealella* could not pupate under a low oxygen environment of 10%. A low oxygen environment of 10% has no obvious effect on the eclosion rate of pupae and pupal period of *T. castaneum*, but it can prolong the pupal period of *O. surinamensis* obviously inhibiting the growth of S. zeamais effectively. Except for certain inhibitory effects on growth and developments of larvae of *S. cerealella*, a low oxygen environment of 15% caused certain prolongations of development periods of other test pests, but had no obvious effect on hatchability, eclosion rate and etc. Low oxygen has an obvious inhibition effect on stored grain pests This inhibition effect is more obvious with the reduction of the oxygen content and prolongation of sealing time, . Therefore, a low oxygen environment of 5% – 10% oxygen can inhibit growths and developments of *T. castaneum*, *O. surinamensis*, *S. zeamais* and *S. cerealella* effectively.

Key words: low oxygen, stored grain pests, population inhibition, life history, different stages

Preface

At present, chemical fumigants are still used extensively as major pesticides for stored products (including stored grains), although they have advantages such as high efficiency, low residual and low cost, their operational safety and environmental safety are of high concern by the public; The rapid increase of drug resistance of the pests[1-4] and the problem of its effect on the ecological environment of the world has become very highlighted [5-6]. For example, the PH_3 resistances of stored product pests is very serious. It results in failure of fumigation. This has been often been reported and threatens the service lives of the fumigants;. Bromomethane's destructive effect on ozonosphere will result in global obsolescence in 2015. Therefore, it is necessary to develop green, environmental friendly and effective newtype fumigants and fumigation technologies. Gas adjustment pests controlling technology means changing the components of gas and their ratios artificially, and thus achieve effective control of pests[7]; It mainly includes reducing the oxygen concentration in an air tight space [8-11] and increasing the CO_2 concentration in the air tight space [12-20], to achieve the goal of pests killing. These technologies belong to the green grain storage technologies which are favored by more and more enterprises, and they have been used in the grain storage industry of our country recently. In order to reduce the application cost the low oxygen grain pests controlling technology has been highly [8-9]. Reducing the oxygen concentration in a grain pile to 2% and below through nitrogen introduction, chemical deoxidizing agents, oxygen-removal by burning or air tight biological oxygen-reducing application for more than 15 days, can kill various stages of grain pests effectively [21]. If the oxygen concentration is more than 2%, there are few reports about the effect on growths and developments of stored gain pests. Although our country has performed film sealing on grain surface and

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five-face or six-face film sealing on a grain pile which is called " double low" or " three low" grain storage technology separately for an extended time, and has found that low oxygen had effects on controlling of pests in grain pile the key parameters for controlling growths and developments of pests such as degree of air airtighness tof the grain pile, oxygen concentration and holding time have not been be determined This influences the extensive promotion of these technologies. The present research was performed to study t the effects of different low oxygen environments on growths and development of four kinds of stored grain pests under the laboratory conditions. They confirmed the need for concentration and time for controlling of the pest population, initially, providing a basis for the establishment of process parameters for controlling of the stored grain pests by low oxygen.

Materials and Method

Materials

Kinds and Sources of Test Pests

The following test pests were cultured in the Academy of State Administration of Grain for dozens of generations *Tribolium castaneum* Herbst (Yiyang strains), *Oryzaephilus surinamensis* Linne (Xinshi strains), *Sitotroga cerealella* Olivier (Xinshi strains) and *Sitophilus zeamais* Motschulsky (Guangzhou strains). Hereinafter, the denotation of test pests are: TC, OS, SC and SZ respectively.

Reagents and Materials

Medical oxygen; purity ≥ 99. 7% ; Beijing Beiwen Gas Production Factory

High-purity nitrogen; purity ≥ 99. 9996% ; Beijing Beiwen Gas Production Factory

Poly tetrafluoroethylene solution; Shanghai Sanaifu New Materials Co. , Ltd.

NaCl; Beijing Chemical Factory

Acid fuchsin; purity ≥ 94% ; Guangdong Xilong Chemical Factory

High activity dried yeast; Angle Yeast Co. , Ltd.

Main instruments and equipments

Electric thermostatic incubator; Shanghai Precision Instrument Co. , Ltd.

Rotary flow-meter, LZB - FB type; Flow rates: 25 - 250mL/Min, 100 - 1000mL/min, 1 - 6L/min; Jiangsu Chemical Engineering Instrument Co. , Ltd.

Vacuum dryer (Φ210mm); Beijing Longyuan Glass Co. , Ltd.

Continuous varyiing power stereo microscope XTS20 series; Beijing Fukai Instruments Co. , Ltd.

Orsat gas analyzer; Shangdong Leling Xinghua Glass Instruments Factory

Culture Plate: self-made. Organic glass of which diameter is 10mm and height is 8mm; there are 54 holes of which diameters are 5mm distributed uniformly and filter paper sticks at the bottom of the cultivation plate, while the covered was made of polyester plate; see Fig 1.

Method

Preparation of Test Pest

Obtaining of the eggs of TC and OS

Place 1000 of TC adults hatched after 2 weeks, into culture bottle and added feed which had passed a 80 - mesh screen (whole wheat flour: yeast, 95 :5). Three days after egg laying, the adults were sieved out with a 40 - mesh screen and remaining feed was sieved with an 80 - mesh screen. Eggs remained on the 80 - mesh screen. The eggs of OS were obtained similarly.

Obtaining the Eggs of SC

500 adults were selected and placed into a culture bottle, the mouth ealed with 18 mesh screen, hung upside down above the egg-receiving culture dish, then placed into an incubator at 30 ± 1℃ and 75% ± 1% relative humidity. After three days, the eggs of adults fell into the culture dish through the 18 mesh screen. The eggs were then placed into the receiving apparatus to perform the test.

Obtaining the Eggs of SZ

500 adults were selected and put into a clean culture bottle with wheat. The bottle was then placed into an incubator for 3 days at a temperature of 30 ± 1℃ and 75% ± 1% relative humidity, The adults were then removed and the wheat was treated with acid fuchsin. Red spots on the wheat, indicated there were eggs.

Preparation of Test

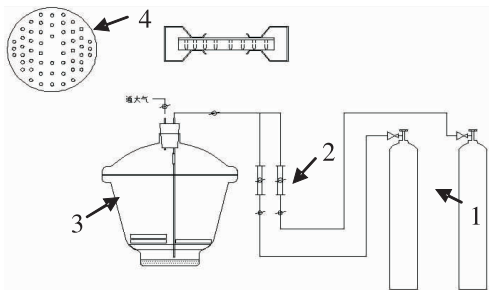
Eggs of TC, OS and SC were sought out the with a small brush the wheat with eggs of SZ were sought out under a stereo microscope and put into the holes of culture plates One test group was used for each oxygen concentration, three repetitions for each group and treatment. Each treatment in the culture plates contained 54 test eggs.

Controlling of the test temperature and humidity

The incubator test temperature was 30 ± 1℃. A saturated NaCl solution o controlled the humidity [2].

Controlling of the low oxygen condition

See Fig 1. According to the different requirements of oxygen concentration for the different tests, oxygen and nitrogen were mixed at different flow rates under the precondition that the outlet pressures of pressure reduction gauge coincided. The mixtures flowed into the dryer (the humidity had been adjusted) in which the test pests had been placed. Tthe outlet gas concentration of the dryer was tested using an Orsat gas analyzer. When the oxygen concentration reached to the desired value and become constant, gas filling was stopped and the outlet. was sealed. The dryer was placed into an incubator of which the temperature had been adjusted. An air – pressure balance bottle with distilled water was used to balance the pressure in the dryer with the pressure outside.



1. gas source; 2. flow – rate controlling device; 3. test container; 4. culture plate

Fig. 1 Schematic diagram of the research equipments for inhibiting of growth and developments of pests by low oxygen

Designs and observations of tests for the treatment group and the reference group

The volume contents of oxygen in the treatment group were 15% ±1%, 10% ±1% and 5% ±1% and the volume contents of oxygen in the reference group was 21% of that in the normal atmospheric environment.

The culture plates were taken out for testing the pests at fixed time every day, and the hatches observed and recorded, Exuviations, pupations and eclosions of the test pests were examined under a stereo microscope. The treatment groups were exposed to the gas environments after observations were finished every day to create the same low oxygen environment, and the dryer then seaed after each inspection.

Dada processing method

An SAS data processing software was used, and analysis of significance was performed with with the anova program. .

Result and Analysis

Inhibition effect of the low oxygen environment on TC, OS and SC in various stages

Under the conditions of temperature of 30 ±1℃ and 75% ±1% humidity is, the development history, hatch rate, pupation rate and eclosion rate of TC, OS and SC are shown in tables 1 to 3.

2.1.1 Hatch and development of eggs

In table 1, we can see that with the reduction of the environmental oxygen concentration, hatch rates of these three test pests were gradually reduced, and were reduced to 0 when the oxygen concentrationreached 5%. When the oxygen concentration was 15%, hatch rates of TC and OS were not effected, but the hatch rate of SC was inhibited. Table 1 also shows that the egg stages of these three test pests were prolonged under the low oxygen environment. When the oxygen concentration was 15%, it prolonged the egg stage of SC. When the oxygen concentration was 10%, the egg stages of TC and SC were prolonged ($a = 0.01$), but the prolongation of the egg stage of OS was not obvious. It is obvious that the low oxygen environments of 15%, 10% and 5% have certain lethal effects on eggs of these three test pests, and with the reduction of the oxygen concentration, the death rates of the eggs increased and reached 100% under the low oxygen environment of oxygen concentration of 5%. At the same time, with the reduction of the environmental oxygen concentration, the egg stages were gradually prolonged.

Table 1. Inhibition effect of the low oxygen environment on the eggs of TC, OS, and SC

O_2 (%)	Hatch rate (%)			Egg stage (d)		
	TC	OS	SC	TC	OS	SC
21	90.7 ±2.0A	75.0 ±5.0A	66.7 ±2.3A	3.5 ±0.1A	4.02 ±0.09A	5.5 ±1.0A
15	86.0 ±1.0A	73.3 ±3.3A	24.1 ±3.9B	3.6 ±0.1A	4.21 ±0.06A	6.7 ±0.5B
10	79.6 ±3.2A	20.0 ±3.3B	23.5 ±4.1B	4.0 ±0.1B	4.30 ±0.15A	7.4 ±0.7C
5	0	0	0	–	–	–

Note: The letter in the table represents the difference is very significant under the level of $a = 0.01$.

Pupation Rate of Larva and Larva Stage

From table 2, we can see that when the environmental oxygen concentrations were 15% and 10%, in comparison with the data of the reference group, the pupation rates of the larvae of TC were inhibited, but there was no significant difference between the results under these two concentrations; For OS when compared with the reference group, the oxygen concentration of 10% inhibited the pupation rates of OS. When the oxygen concentration was 15%, it inhibited the pupation rates of SC, and the larvae could not pupate. when the oxygen concentration was 10%. At the same time, with reduction of the environmental oxygen concentration, the larva stage was gradually prolonged. Therefore, with low oxygen environments of 15% and 10%, the larva stage of TC is prolonged for 1 day and 5 days respectively and there is a significant difference ($a = 0.05$). The effects on larva stages of OS and SC were relatively small. Therefore, the low oxygen environments of oxygen concentrations of15% and 10% have lethal effects on the larvae of these three test pests. With the reduction of the oxygen concentration, the pupation rates of larvae were reduced, i. e. the death rates increased.

Table 2. Inhibition effect of the low oxygen environment on the larvae of TC, OS and SC

O_2 (%)	Hatch rate(%)			Egg stage(d)		
	TC	OS	SC	TC	OS	SC
21	81.7 ±5.1a	97.2 ±1.9a	66.7 ±2.4a	28.3 ±1.7a	21.91 ±0.05a	16.2 ±0.9a
15	50.0 ±7.8b	89.4 ±2.0a	31.9 ±3.1b	29.1 ±1.7a	20.00 ±0.09a	18.1 ±0.9a
10	43.5 ±8.1b	66.7 ±0.1b	0c	33.1 ±1.6a	22.25 ±0.63a	–
5	–	–	0	–	–	–

Note: The letter in the table represents the difference is very significant under the level of $a = 0.05$.

Eclosion Rate of Pupa and Pupal Stage

From table 3, we can see that with the reduction of the environmental oxygen concentrations, the effects on eclosion rates and pupal stages of these three test pests are the same basically as with that the eggs and larvae; eclosion rates are reduced and pupal stages are prolonged. The obvious effect only occurs on pupae of OS treated with low oxygen environment of oxygen concentration of 10%.

Table 3. Inhibition effect of the low oxygen environment on the pupae of TC, OS and SC

O_2 (%)	Hatch rate(%)			Egg stage(d)		
	TC	OS	SC	TC	OS	SC
21	91.4 ±5.1a	85.1 ±2.9a	91.7 ±4.1a	5.3 ±0.3a	4.06 ±0.04a	5.2 ±0.6a
15	91.6 ±8.3a	71.1 ±2.0a	71.4 ±4.6a	5.5 ±0.7a	4.07 ±0.05a	5.9 ±0.6a
10	88.7 ±6.6a	57.1 ±3.1B	–	6.0 ±0.2a	4.50 ±0.50B	–
5	–	–	–	–	–	–

Note: The letter in the table represents the difference is very significant under the level of $a = 0.01$

Effects on Generations of TC, OS, SZ and SC

From Table 4, we can see that with the reduction of the environmental oxygen concentrations, the generation completion rates of these four test pests were gradually educed, and the development times of all the generations were gradually prolonged. . Under the low oxygen environment of 15%, the reductions of the generation completion rates of TC, OS, and SZ were not obvious. The generation completion rate of SC was reduced significantly; by 86. 5%; in comparison with the reference group. There were no significant inhibitions of generations in these four kinds. The generation completion rate of SC was 0 when the oxygen concentration was 10%, and the generation completion rates of other three kinds of the test pests were reduced significantly. Their generation were prolonged ($a = 0.05$). This shows that under the low oxygen environments of which oxygen concentrations are 15% and 10%, the growths and developments of these four kinds of test pests were inhibited, and under low oxygen environments of oxygen concentrations of 5%, these four kinds of the test pests could not complete their life histories. Under the low oxygen environment of oxygen concentrations of 10%, SC also could

not complete its life history.

Table 4. Inhibition effect of the low oxygen environment on the generations of TC, OS, and SC

O_2 (%)	Generation completion rate (%)				Generation (d)			
	TC	OS	SC	SZ	TC	OS	SC	SZ
21	56.3 ± 8.1a	66.7 ± 7.8a	40.8 ± 5.5a	48.0 ± 4.6a	34.7 ± 1.9a	29.91 ± 0.08a	26.9 ± 0.6a	34.2 ± 0.1a
15	37.5 ± 9.9ab	53.3 ± 5.7a	5.5 ± 1.6b	46.0 ± 4.3a	37.3 ± 2.3ab	30.33 ± 0.11a	30.7 ± 0.6a	34.4 ± 0.2a
10	29.6 ± 4.2b	6.7 ± 3.1b	0	27.0 ± 3.1b	41.4 ± 1.6b	31.00 ± 1.00b	–	36.5 ± 0.4b
5	0	0	0	–	–	–	–	–

Note: The letter in the table represents the difference is significant under the level of a = 0.05

Discussion

Many scholars have performed researches on pest killing by low oxygen [23,24]. Low oxygen not only can kill the pests, but also can effectively inhibit the growths and developments of pests. By observation on the development status of SZ under the environments of O_2 being 10%, CO_2 10% and N_2 80%, we found that the whole development period was prolonged by 10 – 11 days [25]. The low oxygen environment of oxygen concentration of 10% can result in the prolongation of the development period of the TC non – adult stage[26]. The research result of this article also shows the lethal effect and inhibition effect on growths and developments of the test pests. The ATP yields of pests are not enough under the low oxygen environment [27,28], and this is more obvious at the stages of hatch, exuviation, pupation and eclosion, since at these stages, the insects have high energy requirements. Thus the test pests accumulate enough energy through prolongation of the development period in the growth process; this may be the major reason for the prolongations of the development periods of the test pests.

Abroad, the process parameters of rapid stored grain pest killing by low oxygen are that when the oxygen concentration is less than 3%, the pests will be killed rapidly (above 15 days)[29], but the reports on the inhibition of growths and developments of stored gain pests by the low oxygen environment are few. The results of the present research are that, under the condition of keeping oxygen concentration in grain pile for a long time, such as keeping the oxygen concentration at 5% – 10% for more than 2 months, there is an inhibition effect on increasing populations of the stored grain pests in the grain pile, or the pests die gradually to realize the goal of pest controlling. Therefore, when low oxygen technology is extended and applied to inhibit stored grain pest populations, airtighness should be enhanced. Research and development should be performed on methods and technologies of keeping the low oxygen concentration in grain pile for a long time. This would reduce the application cost of the technology and stored grain pest control by low oxygen will have good development prospects in our country.

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