International Journal of Latest Research in Agriculture and Veterinary Sciences (IJLRAVS)
www.ijlravs.com
Volume 01 Issue 01 ǁ July – September 2022 ǁ PP. 24-33

Analysis and Research on Insecticidal Effect of 200 mL/m^3 Phosphine Fumigation under Nitrogen-rich and Hypoxia

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Abstract: This thesis mainly studied the fumigation insecticidal effect of 200mL/m^3 phosphine under different hypoxia conditions, the death law of pests and the comparison of drug resistance of different insect species to phosphine. The results showed that the fumigation insecticidal effect of 200mL/m^3 phosphine gas under hypoxia with oxygen content of 2%, 3%, 4% and 5% was significantly better than that of 200mL/m^3 phosphine gas under constant oxygen, and the average corrected mortality increased by more than 80%;When the oxygen content is more than 3%, the corrected mortality rate of the tested insects increases with the decrease of the oxygen content; When the oxygen content decreases to 3%, the corrected mortality rate reaches the maximum, and the corrected mortality rate of the tested insects decreases while the oxygen content continues to decrease;In terms of time, more than 80% of the tested insects in the hypoxia group were killed within 2 days and almost all died within 3 days; The tolerance to 200mL/m^3 phosphine sitophilus oryzae was stronger than that of Rhizopertha dominica under hypoxia.

Keywords: Hypoxic; Phosphine; Fumigation; Corrected Mortality Rate

Food is the basic food on which people depend for survival. However, according to the statistics of the Food and Agriculture Organization (FAO), 10% of the world's food is lost due to insect pests(Ge Lingyan., 2011).As a major food producing country, China loses 5% of its food due to insect pests every year during storage, while the loss of insect pests in the national food reserve accounts for 30% of the total loss(Wang Jinglei et al., 2014).This has caused huge losses to the quantity and quality of our country's food after delivery. Therefore, it is extremely important to control insect pests after delivery (especially in the food reserve).

Fumigation is the most direct and effective method to control pests in food depots. At present, aluminium phosphide is the most widely used fumigant in the field of food fumigation. Its principle is that phosphine gas generated by the reaction between aluminium phosphide and water has a great killing effect on pests. Phosphine has the characteristics of good diffusibility, good volatility and easy penetration into the lower layer of food heap due to its low molecular weight, low boiling point and small gas specific gravity. At the same time, it is easy to apply, has no toxic residue and is safe, making it one of the most widely used fumigants in the world(Lindgren et al.,1958; Halliday at al.,1983; Xiao compiled,2001).However, phosphine has been used in food storage for half a century since 1960s in China.Long-term and large-scale continuous use has made pests

have serious resistance to phosphine, thus increasing fumigation difficulty(Li et al.,2013; Biliaderis et al.,1995).On the other hand, in order to achieve the fumigation effect, the use of phosphine is also increasing, which will bring higher safety risks.

Sitophilus oryzae belongs to Coleoptera, family Elephantidae, and is an insect in Complete metamorphosis. Its life experiences are as follows: egg → larva → pupa → adult. It is the main pest for storing foods. It is mainly parasitic in foods such as corn, rice, wheat, sorghum and flour. Its adult gnaws at food foods and its larva eats inside foods. Because of its rapid growth and widespread damage, its geographical distribution can be spread all over the world, while in China, it is mainly distributed in the south. In recent years, sitophilus oryzaes have caused more serious damage in China (Wang et al.,2014). Rhizopertha dominica is one of the main stored food pests, and its food habits are complex. It can harm cereals, flour, cereals, dried fruits, Chinese medicinal materials and bamboo and wood equipment, among which paddy, wheat and flour are the most serious. Sex prefers warmth and can develop at higher temperatures. Larvae are moth-eating, and adults lay eggs on the surface of food or in food crumbs. The optimum temperature is 34℃, the spawning quantity is high and the development is fast. Adults can't destroy whole rice foods, they can only invade from places with wounds, and can drill to the bottom of food piles. Therefore, they have great destructive power on food with wounds, and the existence of sitophilus oryzaes can intensify their destructive power on food.

Nitrogen-rich hypoxia food storage has incomparable advantages in pest killing, bacteriostasis and mildew prevention, green preservation and reduction of personal injury（Wang et al.,2014; Dale,2008）. Since the end of 1960s, China has carried out indoor research on hypoxia food storage and small-scale warehouse tests. One of the methods is to pump air out of a sealed food pile and fill it with nitrogen, which is called nitrogen-rich food storage. Compared with fumigation technology, nitrogen air control storage technology is green and safe. With the development and popularization of nitrogen controlled atmosphere technology, the application scope of nitrogen controlled atmosphere in China is gradually increasing, and it is considered as a pest control method with relatively low economic cost and reliable effect(Bo et al.,2013). However, nitrogen modified atmosphere has the characteristics of long operation time and high sealing requirement. Generally, it requires more than 98% N_2 ratio and 30 days maintenance. The air tightness requirement for warehouse is much higher than that of CO_2.Otherwise, N_2 needs to be supplemented many times, and its application cost is much higher than that of CO_2. At home, Li Minglong et al. (Li et al.,2013) used 95% mixed gas of nitrogen and phosphine to carry out experiments on corn with high moisture content stored in summer. The results showed that they could keep the inhibition effect on mold for a long time in food piles and inhibit the change of high moisture content corn quality. In this experiment, nitrogen was used to replace oxygen, and the insecticidal effect of phosphine fumigation under different hypoxia conditions was analyzed and studied, including pest mortality rate, death time and tolerance to phosphine, etc. The aim was to explore more efficient insecticidal methods and provide basic data and theoretical reference for the research of new methods in green food storage.

1. Materials and Methods

1.1 Materials and reagents

The tested insect species:sitophilus oryzae resistance (R) and Rhizopertha dominica resistance (R) all come from pest Control Laboratory of Chengdu Storage Research Institute Co., Ltd. of China Food Reserves Corporation

Phosphine:Prepared from aluminium phosphide by drainage method 99% High Pressure Nitrogen:Sichuan Qiaoyuan Gas Co., Ltd.

1.2 Main instruments and equipment

GML-2100 Portable Nitrogen and Oxygen Analyzer:Chang 'ai Electronic Technology Co., Ltd.

LB8-LCD High Precision Intelligent Temperature Control:Zhejiang Longben Electric Co., Ltd.

HNS1803 Heating Heater:Qingdao Haier Household Appliance Service Co., Ltd.

Drager PAC III Phosphine Gas Detector:Drager Safety Equipment Co., Ltd.

Drager Circulation Pump:Drager Safety Equipment Co., Ltd.

Pressure gauge:Chengdu Liangyun Food and Agriculture Technology Co., Ltd.

Glass instruments:2L double-pass glass tube with stopper, 1L beaker, glass rod, gas collector with rubber stopper

1.3 Test method

1.3.1 Preparation and concentration determination of phosphine gas

1.3.1.1 Preparation of phosphine gas

Preparation principle: $AlP + H_2O === PH_3 \uparrow + Al(OH)_3 \downarrow$

Preparation method: collect gas by drainage method, place the gas collector in a 1L beaker, and add water to the beaker until the gas collector is filled with water. Wrap the Aluminium phosphide solid with double-layer gauze, tie it tightly with rubber bands, and fix it at one end of the glass rod. Hold the other end of the glass rod and place the Aluminium phosphide at the bottom of the gas collector, so that the generated gas enters the gas collector upward and the water inside is discharged at the same time. After about 2h, the Aluminium phosphide completely reacts with water, the residue is taken out, and the prepared phosphine gas is used for later use. The whole process is carried out in a ventilated kitchen.

1.3.1.2 Determination of phosphine gas concentration

A 2L double-pass glass tube, a circulation pump and a phosphine concentration detector are formed into a closed circulation loop through a rubber hose, and the air tightness of the circulation loop is measured by a U-shaped pressure gauge to ensure that no air leakage phenomenon occurs. Use a micro syringe to extract 2mL of prepared phosphine gas, inject it into the 2L circulation loop from the rubber tube, turn on the circulation pump, make phosphine gas circulate in the 2L glass tube, and observe the value displayed by the phosphine detector in real time. When the value is stable, the phosphine concentration in the glass tube is 885mL/m3.

1.3.2 Assembly of experimental equipment and measurement of air tightness

Five 2L two-way glass tubes are connected in parallel. Both ends of each glass tube can be clamped with rubber hoses to form an independent space. Finally, the circulation pump and phosphine concentration detector are connected through rubber hoses to form a fumigation device, as shown in Figure 2. At the same time, one end of the U-shaped pipe of pressure gauge is connected into the fumigation device through a rubber hose, and air is injected into the fumigation device through the rubber pipe to pressurize, so that a liquid level difference (pressure difference) is generated on the liquid level of the U-shaped pipe of pressure gauge, and the pressure value of the liquid level difference is recorded. Stop gas injection, seal the gas injection port with a water stop clip at the same time, observe the change of pressure value of pressure gauge, and the pressure value will not change within 1h, proving that the Circulation fumigation device has good air tightness. On the contrary, it indicates that the device has air leakage.Apply soapy water on each interface to find the air leakage point, and

perform encryption treatment until the purpose of good air tightness is achieved.

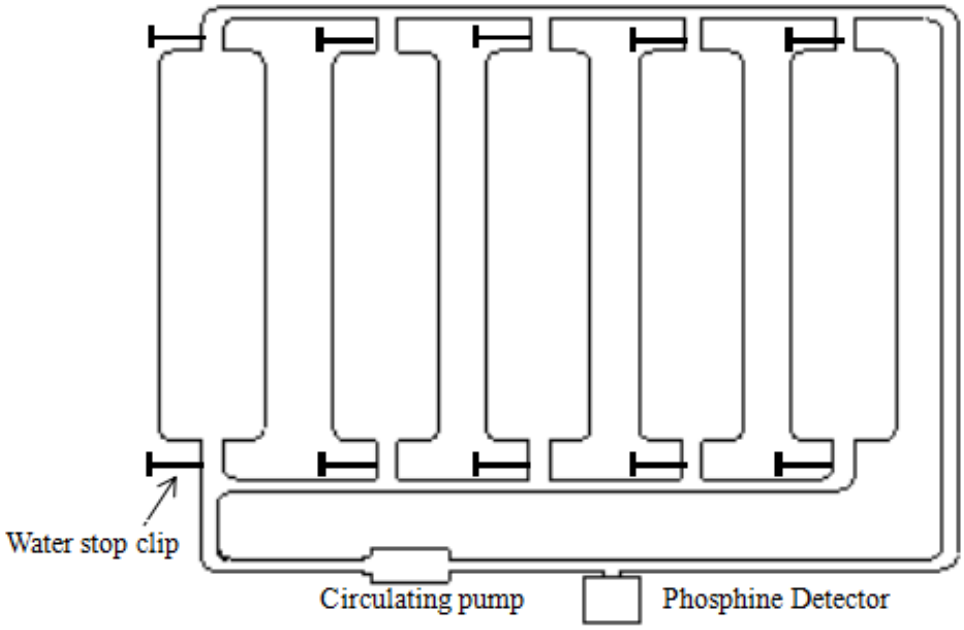


Figure 1 Two-way Glass Tube

Figure 2 Phosphine Circulation fumigation Test Device

1.3.3 Adjustment of different hypoxia environments and injection of phosphine gas

Different nitrogen-rich and hypoxia environments are formed by filling nitrogen to replace oxygen. There are five groups of hypoxia group and control group (normal air) with four gradients. The oxygen content of hypoxia group is respectively set at 2%, 3%, 4% and 5%, i.e. the corresponding nitrogen content is respectively 98%, 97%, 96% and 95% (the rare gas content is ignored). The specific operation method is as follows: open the No.1 pipe water stop clamp, close the No.2, 3, 4 and 5 water stop clamps, charge with 99% nitrogen in the steel cylinder, use the nitrogen detector to detect in real time, and stop injecting nitrogen when the nitrogen concentration in the glass tube is 98% and stable within 3 minutes. Then, turn on the circulating pump, inject phosphine gas gradually, and use phosphine detector to detect in real time. When the concentration

of phosphine gas is stable at 200mL/m^3, stop injecting gas. At this time, tube 1 formed a nitrogen-rich and hypoxia environment with 98% nitrogen and 2% oxygen. With this operation method, different gas environments of the other 4 groups of glass tubes are set in sequence. Three bags of tested insects were placed in each gradient for parallel tests, and placed in a thermostatic chamber at 30℃.After that, one group was taken out every day (24h), and the mortality was counted after the taken out insects were placed at room temperature for 24h.As shown in Table 1, the test plan for 5% oxygen (95%N_2) content is the same for all other hypoxia groups except oxygen content:

Table 1: Test Plan in 5% Hypoxic Environment

Serial number	O_2 content （%）	N_2 content （%）	PH_3 concentration (mL/m^3)	Time(d)	Insect species	
					Sitophilus oryzae R	Rhizopertha dominica R
1	21	78	200	1	3	3
2	5	95	200	2	3	3
3	5	95	200	3	3	3
4	5	95	200	4	3	3
5	5	95	200	5	3	3
6	CK	95	0	5	3	3

Note: CK refers to the tested insects placed in the air at 30℃, which is used to calculate the corrected mortality rate of the test group.

Results and Analysis

The test data were processed and statistically analyzed by Excel2010, SPSS22 and Origin8.0, and the difference was analyzed by paired samples T test.

After 5 days (120h), all the test insects are taken out, and the death situation of the test insects is counted after being left at room temperature for 24h, and the number of deaths is recorded. The calculation method is as shown in formula (1) (2) in terms of corrected mortality rate:

$$\text{mortality rate} = \frac{\text{number of dead insects}}{\text{test insects}} \times 100\% \quad (1)$$

$$\text{corrected mortality rate} = \frac{\text{Test mortality} - \text{Control mortality}}{1 - \text{Control mortality}} \times 100\% \quad (2)$$

2.1 Test Results

For convenience of analysis, A, B, C, D and E respectively represent the control group, 5% hypoxia group, 4% hypoxia group, 3% hypoxia group and 2% hypoxia group.The corrected mortality of different insect species in different oxygen content and fumigation time are shown in Table 2. Meanwhile, the significant difference between the control group and each nitrogen-rich group is tested, and the results are shown in Tables 3 and 4.

Table 2 Average Corrected Mortality Rate of Different Insect Species Injected with Gas in Different N_2 Content

and Time

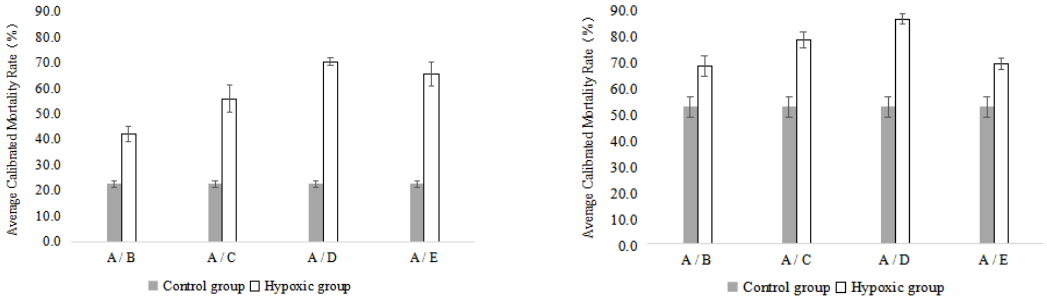
	Average Corrected Mortality（%）									
	Sitophilus oryzae R					Rhizopertha dominica R				
O_2 content	One day	Two day	Three day	Four day	Five day	One day	Two day	Three day	Four day	Five day
21%	22.5	80.9	100.0	100.0	100.0	53.3	90.0	100.0	100.0	100.0
5%	42.2	86.7	100.0	100.0	100.0	68.9	95.6	100.0	100.0	100.0
4%	55.9	88.9	100.0	100.0	100.0	78.9	98.9	100.0	100.0	100.0
3%	70.4	97.8	100.0	100.0	100.0	86.7	100.0	100.0	100.0	100.0
2%	65.6	84.4	100.0	100.0	100.0	69.1	92.2	100.0	100.0	100.0

Note: Nitrogen content indicates the proportion of nitrogen contained in the mixed gas except 200mL/m3 phosphine gas.

Table 3 Significant Difference Analysis between Control Group and Hypoxic Group after Fumigation for 1 Day

Insect species	A / B		A / C		A / D		A / E	
	t	p	t	p	t	p	t	p
Sitophilus oryzae R	-11.579	0.007	-7.618	0.017	-16.735	0.004	-7.210	0.019
Rhizopertha dominica R	-14.364	0.005	-6.386	0.024	-5.773	0.029	-18.555	0.003

The comparison of average corrected mortality between control group and hypoxia group after fumigation for 1 day (24h) with sitophilus oryzae R and Rhizopertha dominica R shown in table 2 is shown in Figure 1, r indicates resistance:



（a） （b）

Figure 3 (a) control group and hypoxia group after sitophilus oryzae R fumigation for 1 day (24h).Mean corrected mortality comparison (b) Control group and hypoxia group after Rhizopertha dominica R fumigation for 1 day (24h).Mean corrected mortality comparison

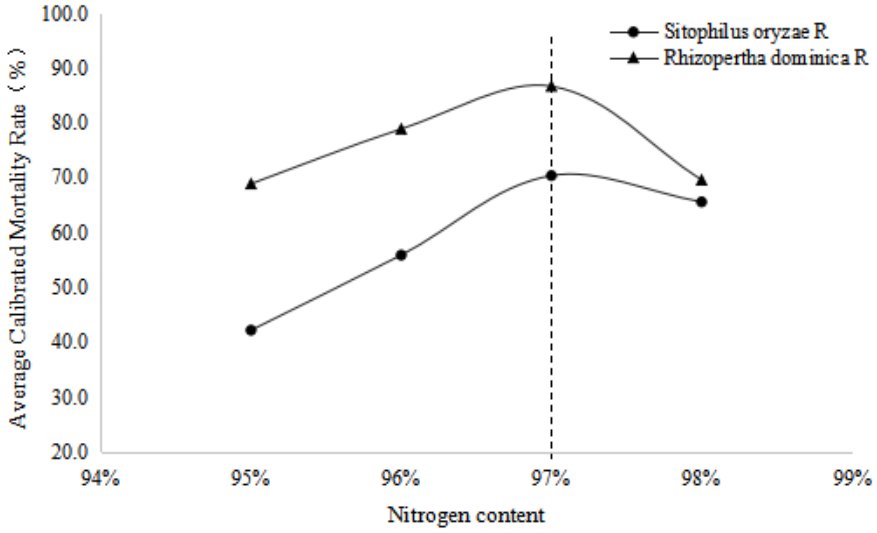


Figure 4 Sitophilus oryzae R and Rhizopertha dominica R increase with nitrogen content (oxygen content decreases). Average corrected mortality changes

As can be seen from table 3 and Figure 1, after fumigation for 1 day (24h), compared with the control group, the average corrected mortality rate of the tested insects in the hypoxia group after nitrogen-rich is significantly increased ($t < 0$), and the difference significance analysis P is less than 0.05, especially when the oxygen content is reduced from 21% (control group) to 5%(N_2 content 95%), $P < 0.01$, the difference is extremely significant, indicating that in a certain range, when the oxygen content is greatly reduced, the mortality rate of the pests is sharply increased. The corrected death rate of sitophilus oryzae r increased by 87.6% and that of Rhizopertha dominicas by 29.3%, and increased with the decrease of oxygen content (as shown in Figure 2), reaching the maximum when the oxygen content was 3%, the corrected death rate of sitophilus oryzae r increased by 212.9% and that of Rhizopertha dominicas by 62.7%. Continue to reduce the oxygen concentration, the corrected mortality rate of the two species of worms began to decline. The mechanism of this phenomenon is still unclear. It has been reported that during the combined use of carbon dioxide and phosphine, a certain amount of carbon dioxide can stimulate insect respiration and reduce the dosage of chemicals and treatment time. However, the higher the amount, the better. After exceeding a certain amount, the effect will decrease (Lu et al,.1996; Manivannan et al.,2016).Nitrogen may have similar properties. With the increase of nitrogen content, the respiration of pests will be stimulated, and the amount of phosphine inhaled within a certain period of time will increase, increasing the mortality rate. When the nitrogen content increases to a certain value, the respiration of pests will be inhibited, and the amount of phosphine inhaled will be relatively reduced, thus causing the mortality rate to decrease.

In addition, in the control group, the corrected mortality rate of sitophilus oryzae R in each hypoxia group after fumigation for 1 day (24h) was not more than 25%, 22.5%, while the corrected mortality rate of Rhizopertha dominica R was more than 50%, 53.3%;Similarly, the corrected mortality rate of sitophilus oryzae R was significantly lower than that of the Rhizopertha dominica R in the hypoxia group, indicating that the killing effect of 200mL/m^3 phosphine on the Rhizopertha dominica was better than that of sitophilus oryzae, i.e. sitophilus oryzae was more resistant to 200mL/m^3 phosphine in the constant oxygen and hypoxia environment.

Table 4: Significant Difference Data Table after Fumigation for 2 Days

	A / B		A / C		A / D		A / E	
	t	p	t	p	t	p	t	p
Sitophilus oryzae R	-2.679	0.116	-1.823	0.210	-9.568	0.058	-1.965	0.190
Rhizopertha dominica R	-1.000	0.423	-0.385	0.737	-1.000	0.423	-0.385	0.737

The average corrected mortality of control group and hypoxia group after fumigating for 2 days (48h) with sitophilus oryzae R and Rhizopertha dominica R shown in table 2 is compared, such as Figure 3

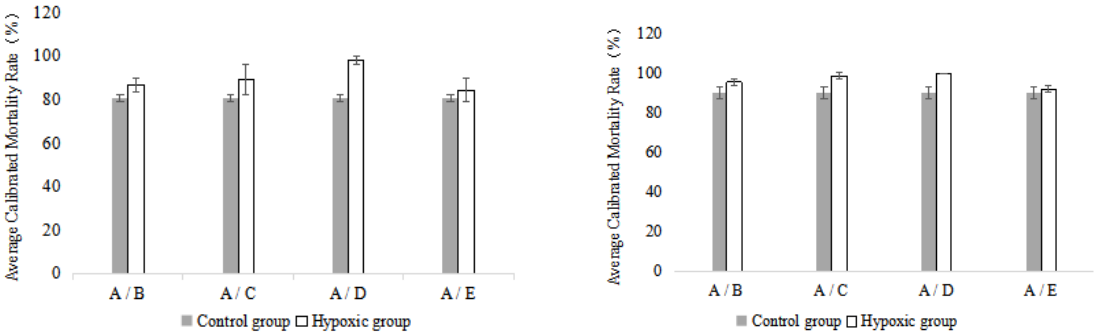


Figure 5 (c) shows the control group and hypoxia group respectively after sitophilus oryzae R fumigation for 2 days (48h).Mean corrected mortality comparison. (d) Control group and hypoxia group after Rhizopertha dominica R fumigation for 2 days (48h). Mean corrected mortality comparison

As can be seen from Table 2 and Figure 3, after fumigation for 2(48h), the average corrected mortality rate of the two tested insects in the control group exceeded 50%, and compared with sitophilus oryzae, the corrected mortality rate of Rhizopertha dominica is higher, with the maximum of Rhizopertha dominica s reaching 100%;The average corrected mortality rate of the two tested insects in the hypoxia group was over 80%, and the mortality rate of Rhizopertha dominica also reached 100%. As can be seen from Table 4, the significant difference between the control group and the hypoxia group was analyzed and found to be $P > 0.05$, i.e. the difference between the control group and the hypoxia group was not significant after fumigation for two days, indicating that Nitrogen-rich hypoxia fumigation has more significant insecticidal effect in a short time (less than 24 hours), and is more conducive to rapid insecticidal in a short time, thus avoiding adverse effects caused by long-time application.

In addition, it can be seen from Table 2 that the average corrected mortality rate of the two tested insects reached 100% by the third day of fumigation, i.e. all tested insects were killed after three days of fumigation, which can provide reference for the longest application time of fumigation of sitophilus oryzae and Rhizopertha dominica.

Conclusion

The fumigation effect of 200mL/m^3 phosphine gas in a hypoxia environment filled with more than 95% nitrogen is obviously better than that of 200mL/m^3 phosphine gas alone, which shows that the former increases

the average corrected mortality rate of pests by more than 80% in the same time.

In the process of nitrogen-rich and hypoxia fumigation, the nitrogen content starts from 78%, the corrected mortality rate of the tested insects increases with the increase of nitrogen content, the corrected mortality rate of the tested insects reaches the maximum when the nitrogen content increases to 97%, and the mortality rate of the pests decreases when the nitrogen content continues to increase to 98%.The mortality rate of the pests is reduced, indicating that the nitrogen-rich and hypoxic conditions can inhibit the respiration of the pests under certain conditions, thereby reducing the effect of fumigation and insecticide;

In terms of time, more than 80% of the tested insects in hypoxia group were killed within 2 days and all died within 3 days. For Rhizopertha dominica R, after fumigation for 1 day (24h), the average corrected mortality rate in the control group was the lowest 53.3%, while that in the experimental group was the lowest 68.9%. After the 2nd day (48h), the average corrected mortality rate in the control group was the lowest 59.6%, and that in the experimental group was the lowest 85.4%. All died on the 3rd day. For sitophilus oryzae R, the corrected mortality rate was lower after the 1st day of fumigation, the control group was 22.5% lower than the control group, and the hypoxia group was 42.2%. After 2 days of fumigation, the corrected mortality rate increased significantly. The sitophilus oryzae R in the control group was the lowest 80.9%, while that in the hypoxia group was the lowest 86.7% under the condition of 5% hypoxia, and all died on the third day.

For different tested insect species, the resistance of Sitophilus oryzae to 200mL/m^3 phosphine in constant oxygen and hypoxia environment is stronger than that of Rhizopertha dominica, i.e. Rhizopertha dominica is easier to be killed under this condition.

Acknowledgements

The authors would like to thank“National Key R&D Program，2016YFD0401004，Research and Development of Key Technologies and Equipment for Green Control of Easy Storage Grain Pests”and “Young Elite Scientists Sponsorship Program by CAST, YESS, 2018QNRC001”.

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