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Variation of Corn Weevil *Sitophilus Zeamais* Population in a Hermetic Preservation Minienvironment Depleted of Oxygen

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Abstract: ***The growth of a corn weevil SITOPHILUS ZEAMAIS was studied in the weevils culture and maize storage minienvironment, with diferent oxygen concentrations ranging from 0%, 5% 10% 15% oxygen, and in the control environment with 21% oxygen. In the weevils culture ME with 21% oxygen the maize weevils SITOPHILUS ZEAMAIS normally growth and there was no dead weevil during the 80 days of experiment, and the percentage loss of corn weight was over 29%. However, under the same experimental conditions, with a lower oxygen concentration, of 15%, 10% and 5%, the number of weevils appeared decreased and the number of dead weevils increased, and the weight loss rate of maize significantly decreased, relatively as a function of the oxygen concentration. In ME at 5% oxygen, after 80 testing days there were 90 weevils appeared, however of which 60 was died, accounting for 67%; in comparison with that at 21% oxygen there were 576 weevils appeared but no death one; the maize weight loss was 5.50%, equal to about only 1/6 of that at 21% oxygen. It was found that, in the test fram, the lower the oxygen concentration in the ME, the lower the number of weevils appeared, the larger the number of death weavils, and the greater the reducing ratio of maize mass loss. In ME with 0% oxygen concentration there was not any weevil appeared and the corn weight loss ratio was just 0%.***

Keywords: Maize weevil, *SITOPHILUS ZEAMAIS*, oxygen depleted minienvironment, testing weevils culture.

1. Introduction

Maize is one of the most important foods, in terms of weight only after rice. In some countries maize is also the most important sources of food [1, 2]. However, at present, the preservation of maize is still inadequate, causing great losses due to oxidation and quality degradation, and mold contamination, including mytoxin molds, which are harmful to human health. and very large loss due to insect pests [3, 4, 5, 6]. Some traditional preservation methods are laborious, of low quality, and economic efficiency. The method of chemical preservation has actually caused many concerns about environmental pollution and serious impact on human health. [5, 6, 7].

Preserving grain corn in hermetic mini-environment (hereinafter referred to as ME) condition with low-oxygen concentration possesses many effects: i- reducing respiration and slowing oxidation deterioration of corn mass [7, 8, 9]; ii- making aerobic organisms, including fungal species, including toxic ones such as mytoxin, difficult to grow and develop, thereby increasing the quality and food safety & hygiene; iii- Pests, especially the maize weevil *Sitophilus zeamais* (Motschulsky), the main pests of storred maize, are deficient in oxygen and can suffocate event to death [7].

Sitophilus zeamais (Motschulsky), abbreviated *S. zeamais*, is one of the typical pests for many types of cereal grains in storage warehouses (10, 11, 12, 13). In particular, *S. zeamais* species easily penetrate and damage maize not yet harvested even in the field, then continue to breed and grow in storage warehouses. In Vietnam, in addition to grain corn, there are other cereal grains such as rice and beans that can be infected with *S. zeamais*, which is one of the most dangerous pests, significantly reducing both quantity and quality of cereal grain after harvest (14, 15, 1, 17, 18). Moreover, for seeds in storage, infection with *S. zeamais* causes the seed to completely lose its ability to germinate [18].

S. zeamais have a long life span of up to 140 days, almost constantly present in the warehouse, even without food as nuts. Under the conditions of adequate food such as in storage space, an adult continuously lays eggs, resulting of a weevil with the all development stages from eggs, larvae, nymphs and adults (18, 19, 20).

Recent research results show that of the five major maize weevils, *S. zeamais* is the most common, accounting for 57.0% of the maize weevil [18]. Therefore *S. zeamais* weevil has been identified as the main pest, typical for corn grain pests. The increase in the number of *S. zeamais* weevils has led to an increase in food demand, an increase in the number of chipped seeds, and a mechanical loss of storred maize. On the other hand, the weevil excretion also reduces the quality of maize, contributing to loss of weight and quality of maize.

Results of research on preserving tens of thousands of tons of rice stored in an air-tight, low-oxygen ME (the oxygen concentration in ME is always lower than 2%) shows that after 24 months of preservation, the quality of rice and the flavor of cooked rice remain almost intact, no mold yeast appears, harmful insects do not grow and develop, the weight loss decreases by less than 1% [5]. Preserving maize in an oxygen-poor environment showed that the corn nutrient content (gluxit, lipit, protein) after 18 months was not significantly reduced (approximately 0%), no fungus ni mold were observed at low oxygen field of 0% [7, 8, 21, 22]. However, there have been no quantitative studies on the effect of oxygen concentration on the growth and development of maize weevils, in particular *S. zeamais*.

This paper presents the results of research on the effect of oxygen poor preservation on the growth and development of the Sitophilus zeamais (Motschulsky) - *S. zeamais*, which contributes to reveal the scientific basis for the selection of methods to preserve maize for a long time, prevent loss effectively and appropriately, non-toxic and food safety and hygiene.

2. Materials and Methods

2.1 Materials and equipements

Laboratory materials: PET from polyethylene terephtalate, transparent tank capacity of 15 liters, locking valve; F1 NK7328 corn kernels from Son La, with a moisture content of 15%; FOCOAR oxygen absorber, in the form of dark pigments, is packed in size of 20x20x5 mm, weight of 30g / pack (ITT provided), plastic jars of height dimension - mouth diameter = 120 - × 30 mm , have a ventilating diaphragm but do not allow the larvae to pass; monofilament.

The grain moisture meter is Farmcomp - Wile 55, error of ± 0.5%; Hygrometer, error of ± 1%; Oxygen meter (measuring percent of oxygen in air, ± 0.2% error), connected to a computer, is home made at VAST. The mini-environment humidity meter is Dual temperature type; raising insect chambers.

Maize grain samples from Son La provinve that were normally stored 6 months after harvest, were considered natural samples, infected with weevils and had a certain amount of weevils eggs available. On average, each 100g of maize contains 56.5 ± 1.5 weevils *S. zeamais*. The identification of maize grain weevils is done according to the document [12], the identification results and classification have been introduced previously [18].

Arrangement of research experiments: Oxygen reducer (1) is placed at the bottom of the bottle, with a plastic stopper to adjust the mouth of the bag to control the concentration of oxygen from outside. An oxygen probe is located in the device, connected to an oxygen meter (Figure 1).

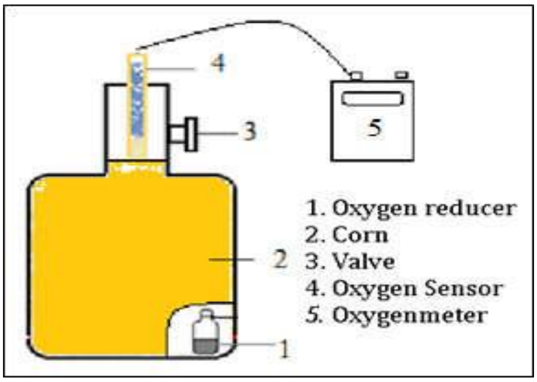

Figure 1: Schemaof typic preservation mini environment

2.2 Experiment procedure

The samples of corn kernels are mixed to screen, removed of impurities, broken grains and the grains of unsatisfactory quality, removed of termites and insects (the procedure is according to TCVN and QCVN [23]). Then the samples were determined the main properties (number of grains, mass, moisture content, color and sympathetic morphology).

Next, the sample was put into a plastic jar with the height dimension and mouth diameter of 120 and 30 mm, respectively, with a weevils membrane but aeration with experiment ME. Each of the three jars is a sample, each of which is connected by a string - tied in a chain. Each vial chain is put into an airtight ME, forming insect culture chambers, the air oxygen content of the MEs is 0%, 5%, 10%, 15%, and the control 21%. These MEs are checked for oxygen concentration, temperature and humidity. The temperature is maintained at 25 °C.

After every 10 days, take the sample of 3 plastic jars containing 90g of corn kernels to tally the number of weevils, and then to remove the faeces, broken grains and loose dust. The remaining weight of maize sample was indexed Wi. Experimental period was 80 days, with 8 counts.

The percentage loss of mass W_i is calculated by the formula:

$$\Delta W_i(\%) = 100\frac{W_O - W_i}{W_O}$$

In which:
W_O: dry matter mass of the original sample
W_i: dry matter mass of the ith collected sample
ΔW_i: Loss ratio of dry matter mass of the ith sample collection
i: index denoting the ith sampling, with i = 1, 2, ... 8

3. Results and Discussion

Variation of weevils population

The results of the inventory of *S. zeamais* weevils in culture EMs with oxygen concentrations of 0%, 5%, 10%, 15%, and 21%, including the number of dead weevils, during the 80-day experiment, are summarized in table 1.

Table 1: Number of *S. zeamais* weevils in air-tight culture media with different oxygen concentrations

T (ds)	Oxygen concentration (%)												
	0	**5**			**10**			**15**			**21**		
		A	D	T	A	D	T	A	D	T	A	D	T
0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	12	0	12	6	0	6	15	0	10	6	0	6
20	0	10	2	12	15	1	16	20	0	20	35	0	35
30	0	12	3	15	36	9	45	54	0	54	96	0	96
40	0	13	15	28	49	20	69	78	0	78	123	0	123
50	0	15	18	33	54	21	75	105	3	108	132	0	132
60	0	30	21	51	60	30	90	123	3	126	231	0	231
70	0	39	39	78	63	33	96	189	12	201	420	0	420
80	0	48	42	90	76	42	118	235	15	250	540	0	540

Under the same conditions of 25^{o}C and 70% moisture content, after 10 days of testing, the number of weevils present in the EMs did not differ significantly. However, the number of weevils that appeared after that in the EM with oxygen concentration of 21%, increases more rapidly than that in the other EMS: after 20 days it was 3 times higher than in EMs of 5%, 10% and about 2 times higher than in EM of 15%. However in the EM of 0% oxygen there did not appear any weevil.

The longer the storage period, the more distinct the number of *S. zeamais* in the ME are. After 80 days at ME of oxygen concentration 5% the total number of *S. zeamais* were 90, and at 10% of oxygen ME there were 118, at 15% of oxygen ME there were 250, and at 21% of oxygen ME there were up to 540 (Table 1, figure 1).

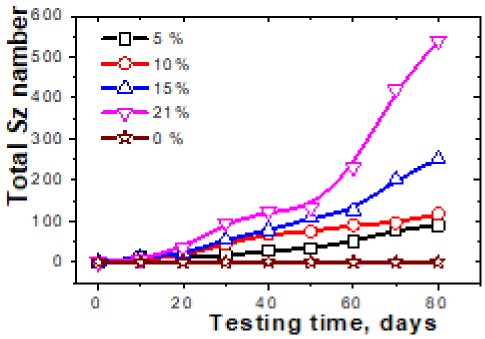

Figure 1: An increase in the total number of weevils over testing time

Thus, it can be seen that reducing the oxygen concentration to low significantly reduces the occurrence of *S. zeamais*. Especially, oxygen 0% ME during 80 days of experiment did not appear any weevil. This result is consistent with previous research, identifying the most suitable ME for preserving agricultural products especially maize grain [5, 6, 7].

The results of determining the number of weevils that appear but being dead in the ME at each the time of the weevil counting test are also remarkable (Figure 2).

In the ME with 5% oxygen concentration, after 20 testing days, there were 2 died weevils of total 12 weevils. After 30 days were 3 dead weevvils / 15. After that, the number of dead weevils increased rapidly, to 42 dead / 90 after 80 days of experiment. Figure 2 shows that in the ME of 5% and 10% oxygen concentration, the numbers of dead weevils are the highest in comparison with the rest MEs.

Note that in MEs with oxygen concentration of 5% and 10%, the number of dead weevils is quite similar, can be considered to be equivalent to each other, after 20 days of experiment in there are 1 and 2 dead weevils, respectively, especially after 80 days the number of dead weevils in both MEs was same 42. However, the total numbers of weevils present in the two environments were different (Fig. 1), so there was a large difference in ratio ofdead weevils / total appeared weevils. After 20 days of experimenting, this ratio was 2/12 and 1/16 respectively, after 50 days were 18/33 and 21/75, and after 80 days this ratio was 42/90 and 42/118. It can be said that those are two transition oxygen concentrations, from relatively high concentrations,> 10%, to relatively low concentrations, <5%, so the number of survival weevils *S. zeamais* varies greatly, the ratio between the number of dead weevils and the total number of weevils appeared is high. With oxygen concentration ≥15%, this rate is low due to the low number of dead weevils, especially in ME with oxygen concentration of 21% this ratio is 0 because there is no dead weevil. It isintresting that this ratio at 0% oxygen concentration is equal also to 0 but due to another reason, that because of no weevil appearing. In Figure 2, the cuvesof dead weevils at oxygen concentration of 0% and 21% are overlap and both equal to 0.

The fluctuations in the number of weevils *S. zeamais* that appear in the MEs at each tallying time after 80 days of the experiment also significantly depends on the oxygen concentration, Figure 3. In ME with oxygen concentration of 21% the number of alive weevils *S. zeamais* increases rapidly, from 6 individuals after 10 days to 35 individuals after 20 days, and after 30, 40, 50, 60, 70, 80 days the numbers are 96, 123, 132, 231, 420 and 540, respectively. In the MEs with oxygen concentration of 15% the number of weevils *S. zeamais* alive also increases significantly as a function of testing time, but much lower than that in ME at 21% oxygen, after 80 days the number of weevils alive was just 235, about 43% in comparison with that at 21% oxygen. In the ME with oxygen concentration of 10% the number of weevils alive decreased sharply, only 6 weevils alive being found after 10 days of experiment, and jusst 76 weevils alive after 80 days. The number of weevils alive in ME at 5% oxygen,after 80 testing days, was only 48. There was not any weevil being appeared in ME at low oxygen concentration of 0%, even dead. Thus, in the ME at 21% oxygen the number of weevils alive is the highest. In general this number of weevils appeared and alive in the MEs decreases with the oxygen concentration.

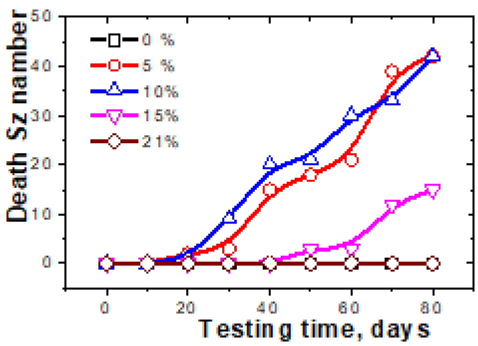

Figure 2: Dead weevil *S. Zeamais*number in experimental MEs over time

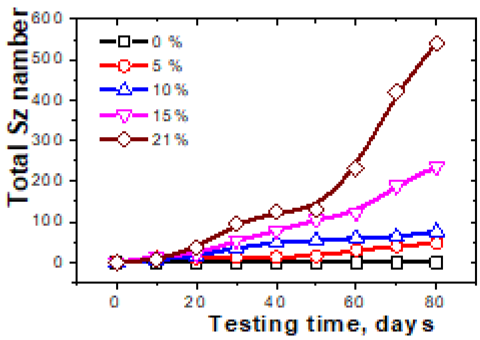

Figure 3: Variation of number of weevils alive in the test ME as a function of experiment time

Weight loss of tested maize

There have been many studies on maize damage and loss caused by insect pests and almost all have confirmed that the maize damage and loss caused by insect during storage are very significant, but have not yet quantified the percentage of net loss, expecialy caused by weevils. On the other hand, nutrient oxidative losses, such as carbohydrates, lipids and proteins, are also considerable, especially over long-term storage, up to 18 months [7, 8, 21, 22]. Short-term storage (only nearly 3 months in this study) significantly limits respiration - oxidation [7] soin this case we can consider the extent of the maize damage due to oxidation is negligible.

The result of 8 times of weight loss determination of tested maize after every 10 days during 80 days of experiment is shown in Table 2 and graphically represented in figure 4.

It is visibly clear that in ME with oxygen natural concentration of 21% the maize loss in weight í very large, figure 4. After 80 days of experiment, the weight loss ratio was catastrophly high, even of 29.20%. This loss percentage is extremly enorme in comparison with that of previous studies, where the cause of maize damage and loss was just oxidation [7, 8, 21, 22].

At oxygen concentration of 15%, the loss ratio decreases to about 1/3, of 10.9% comparably. At lower oxygen concentration, 10% and 5%, the loss ratia are 6.30% and 5.50% respectively. Particularly in ME with oxygen concentration of 0% the original corn weight did not change.

Generally, Comparing to previous long-term experimental results [7, 8, 21, 22] shows that the loss of maize mass, during the short 80-day storage period, is mainly due to a living action of weevils, partly as food for weevils, partly broken apart and degraded due to the process of punching, gnawing on corn kernels and crushing to feed and excretion.

Table 2: Loss in weight (%) of preserved maizeduring 80 days in the culture ME with different oxygen concentration

t, ds	Oxygen concentration (%)				
	0%	**5%**	**10%**	**15%**	**21%**
0	0	0	0	0	0
10	0	0	0	0	0
20	0	0.10	0.18	0.26	1.11
30	0	0.21	0.63	1.02	2.04
40	0	0.40	1.53	3.00	4.25
50	0	3.60	3.74	5.28	9.66
60	0	4.12	4.65	6.42	12.42
70	0	5.16	5.70	8.88	17.94
80	0	5.50	6.30	10.9	29.20

Thus, it can be seen that under environmental conditions with high oxygen concentration of 15 - 21%, a fast growing of weevils causes great loss of mass. However, reducing oxygen levels to limit the growth of the weevils makes sense in the prevention of warehouse insects, in this case is weevils *S. zeamais*, without the use of any toxic chemicals. Especially, the ME without oxygen, with oxygen concentration of 0%, is the preservation environment with the lowest number of weevils, and at the same time causing the lowest percentage loss of maize. This result is consistent with previously published results [7, 8, 21, 22].

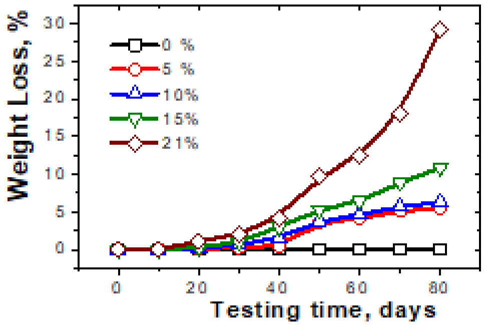

Figure 4: Variation of weight loss ratio of maize grain (%) preserved in culture ME at different oxygenconcentration

4. Conclusion

After 80 days of experiments on preserving maixe and cultivating weevils *S. zeamais*in an air-tight - oxygen-poor VMT with different oxygen concentrations, the results of the experiment showed that the oxygen concentration significantly affected the increase in the occurrence and development of the weevils over time, as well as survival and mortality ratia, and especially to a loss in the storage of maize grain stored.

The ME of 21% oxygen concentration is a favorable condition for a strong growth of weevils, after 80 days of experimenting, the total number of weevils appeared very high, up to 540, the number of dead weevils was just zero and the weight loss was enorme up to 29.20%.

With the oxygen concentration decreasing, to 15%, 10% and 5%, the number of weevils appeared also decreases; In ME at 5% oxygen, there were only 90 weevils *S. zeamais* appeared, however of which 60 was died, accounting for 67%; the maize weight loss was 5.50%, only equal to more than 1/6 in comparíon with that at 21% oxygen. It can be said that the lower the oxygen concentration in the ME, the greater the increase in the number of weevils and the greater the reduction in maize mass loss.

Particularly, in ME with 0% oxygen concentration there did not appear any weevil and the corn weight loss ratio was just 0%; so this ME was selected as the most suitable to preserve maize grain in the long term for prevent of weevil and of weight loss.

5. Acknowledgments

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