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TREATMENT OF THE COWPEA WEEVIL *CALLOSOBRUCHUS MACULATUS* F. (BRUCHIDAE : COLEOPTERA) WITH LOW OXYGEN ATMOSPHERES CONTAINING CARBON DIOXIDE

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Abstract

Controlled atmosphere were investigated as a substitute for conventional stored grain products. The effect of different gas mixtures containing different levels of carbon dioxide against adults and developmental stages of *Callosobruchus maculatus* at 25°C and 70% R. H. was studied. The adults of this species were more susceptible than the other stages to the tested modified atmospheres. Exposure to 75% CO_2 in air for three days was sufficient to cause 100% mortality for eggs, larvae and pupae, whereas 50% CO_2 was sufficient to kill 100% of adults.

INTRODUCTION

The cowpea weevil, *callosobruchus maculatus* F., is one of the most destructive pests of stored leguminous seeds in Egypt. This pest does not confine its attack to leguminous in storage, but also lays its eggs on the exterior of ripening

pods in the field and on seeds in split pods as well.

In recent years, the increase of stored grain and grain products losses as a result of insect infestation had led to a great interest for the use of control measures, chemical and non-chemical. The use of chemicals for grain preserved for human or animal consumption is an unfavourable step towards the protection of such materials. Therefore, urgent need for acceptable and effective methods for preventing insect damage and contamination of stored grains is advisable. One of these methods is the so called modified atmosphere.

This study presents the effects of different gas mixtures of the normal constituents of the atmosphere on different life stages of *C. maculatus* at 25 ± 1°C and 70 ± 5% R. H. exposed for 3 days.

MATERIALS AND METHODS

The parental insects were obtained from the Stored-Product Insects Research Section, Ministry of Agriculture. Stock cultures were set up by introducing 200-400 adults into a two pounds glass jar, half filled with broad beans and covered with muslin, held in place by rubber bands (Strong *et al.*, 1968). The adults were permitted to oviposit in these cultures throughout their life span, which did not exceed 12 days, and then removed, leaving the eggs glued to the surface of the beans. The emerging adults were collected daily and stored in storage jars until used for starting the cultures.

The exposure tests were carried out on 2-days old eggs, one-week old larvae, 3-days old pupae and 2-days old adults. Because the larvae and pupae of *C. maculatus* are living inside the seeds, exposure tests on these stages were carried out in samples of faba bean seeds containing a definite number of them.

Two-pound glass jars were half filled with faba bean seeds. In each jar, about 300 newly emerged adults were released and left to deposit eggs for 2 days, after which they were removed. Samples of bean seeds that held 2-days old eggs were chosen from these cultures and were used in exposure tests for eggs. Other samples

of seeds with eggs attached to them were left for 4-5 days for hatching. Hatched eggs were observed, and the beans were kept until used in exposure tests when the larvae and pupae inside the beans reached the appropriate age.

The experimental unit was 15 seeds having a definite age. This unit was prepared in a glass tube, 7 cm long and 1.5 cm diam., covered in both ends with gauze. These tubes, each containing one stage of *C. maculatus*, were introduced into a gas-washing bottle (dressel flask).

Six gas mixtures were used for treatment of the different stages of *C. maculatus*. These mixtures were :

1. 17% O_2, 68% N_2 and 15% CO_2.
2. 14% O_2, 56% N_2 and 30% CO_2.
3. 11% O_2, 44% N_2 and 45% CO_2.
4. 9 % O_2, 36% N_2 and 55% CO_2.
5. 7 % O_2, 28% N_2 and 65% CO_2.
6. 5 % O_2, 20% N_2 and 75% CO_2.

Bean seeds with different developmental stages and adult stage in gastight connected dressel flasks were held in a constant temperature room at 25°C and were exposed to the gas mixtures by leading gas out of the gas cylinder through copper tubes and a humidifying unit at 70% R. H., consisting of saturated $NaCl/H_2O$ solution into the flasks.

At the outlet of the flasks, O_2 content was determined continously by oxygen analyzer. After about 15 minutes (time for about 10 replacements of total flask volume by gas mixture) outlet concentration was identical with inlet concentration. The gastight sealed flasks were then separted from the gas mixture cylinder to permit further experiments with other flasks. After 3 days as an exposure period, flasks were aerated and seeds were transferred to petri-dishes (15 cm diam.) in a culture room and examined for emerged adults at 25°C. Each sample was accompanied by an untreated control. Experiments were repeated three times.

In order to determine the mortality of adults, the insects had to be examined after 48h to detect any delayed effects, and mortality counts were corrected according to Abbott (1925). For the other developmental stages, mortality was

assessed by counting the emerged adults based on the count of untreated emerged adults.

RESULTS AND DISCUSSION

The results of mortality for the different stages obtained at 25°C and six gas mixtures are presented in Table 1. The mixture containing 75% CO_2 for 3-days exposure induced 100% mortality for all the developmental stages. 100% mortality for adults was produced from the mixture containing 55% CO_2.

The probit analysis based on the computer program indicated by Noack and Reichmuth (1978) revealed that the larvae were more tolerant than the other stages at the LC_{50} level, but the eggs were less susceptible than the other stages at the

Table 1. Percentage mortality of eggs, larvae, pupae and adults of *C. maculatus*, exposed to six gas mixtures at 25°C for 3 days.

Gas No.	Gas mixture (%)			(%) Mortality of treated stages			
	O_2	N_2	CO_2	Eggs	Larvae	Pupae	Adults
1.	17	68	15	15	10	15	25
2.	14	56	30	50	45	42	62
3.	11	44	45	67	72	60	90
4.	9	36	55	86	85	90	100
5.	7	28	65	95	94	96	100
6.	5	20	75	100	100	100	100

LC_{99} level. The adults were the most sensitive stage at the LC_{50}, LC_{95} and LC_{99} levels (Table 2).

Generally, high CO_2 contents were relatively more toxic for all developmental stages of *C. maculatus* than low CO_2 contents. These results are in agreement with the findings of Jay (1980), who found in his studies on *C. maculatus* that 62% CO_2 gave over 90% mortality to 0-24-hour old eggs at 2-days exposure. Storey (1975) also found that the earlier and later stages of *Sitophilus oryzae* were more susceptible and the intermediate stages were less susceptible to the tested modified atmospheres (1% O_2, 8.5% and 11.5% CO_2) when exposed at 21°C and 27°C. At 27°C, 95% mortality of the 4th larval instar through early pupal development occurred after about 10 days for *Sitophilus oryzae*. Reichmuth (1988) indicated that the lethal exposure period was 6 days to achieve 95% mortality with adults of *S. granarius* at 3% O_2 and 20°C. Navarro, (1978) found that the immature stages were the most tolerant to the tested modified atmospheres. However, a 5 days

Table 2. Lethal concentration levels of CO_2% for different stages of *C. maculatus* at 25°C and 3-days exposure.

Stage	LC level (%)		
	LC_{50}	LC_{95}	LC_{99}
Egg	27.95	55.41	73.57
Larva	29.28	55.86	72.52
Pupa	28.51	55.16	72.52
Adult	21.82	38.41	48.54

exposure at 27°C was sufficient to cause 100% mortality of *Oryzaephilus surinamensis*. Also, HASHEM and Reichmuth (1992) mentioned that 40% CO_2 content for 5 days exposure were sufficient to kill 100% of 0-24-hour eggs of *Ephestia elutella, Corcyra cephalonica* and *Sitotroga cerealella* at 25°C.

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تأثير تعريض حشرة خنفساء اللوبيا *Callosobruchus maculatus* F. لجو محدد المحتويات الغازيه (منخفض فى محتواه الأكسجينى ومرتفع فى محتواه من ك أ٢)

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أجريت دراسات على تخزين الحبوب فى جو محدد محتوياته الغازيه كطريقه بديله عن الطرق المتعارف عليها فى مقاومة حشرات المخازن. فقد تم دراسة تأثير تركيزات مختلفه من غاز ك أ٢ فى مخاليط الهواء على الحشرات الكامله لخنفساء اللوبيا *Callosobruchus maculatus* وكذلك أطوارها الغير كامله عند درجة حراره ٢٥م ورطوبه نسبيه ٧٠٪.

وقد بينت الدراسات أن الحشره الكامله هى أكثر الأطوار حساسيه للتعرض لهذه التركيزات وأن التعرض لتركيز ٧٥٪ ك أ٢ فى مخلوط الهواء لمدة ٣ أيام كافى لإحداث ١٠٠٪ موت لكل من أطوار البيض واليرقات والعذارى. بينما التعرض لتركيز ٥٠٪ ك أ٢ فى مخلوط الهواء كان كافياً لإحداث ١٠٠٪ موت للحشرات الكامله.