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*Full Length Research Paper*

# The effect of carbon dioxide at high pressure under different developmental stages of *Callosobruchus maculatus* (F.) hosting on chickpeas

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**In this paper, the minimum exposure time required to kill *Callosbruchus maculatus* (F.) in all the developmental stages of 99.90% purity carbon dioxide ($CO_2$) under 20 bars at 23 to 26°C was determined. Adults, pupae, larvae and eggs of *C. maculatus* hosting kabuli type organic chickpeas were exposed to $CO_2$ for 240 min. The minimum exposure time for killing *C. maculatus* was found to be 180 min in all its developmental stages. As a consequence, one minute exposure time was sufficient to kill the adults of *C. maculates*. However, it was determined that the pupae stage was more tolerant to high pressure $CO_2$.**

**Key words:** *Callosabruchus maculatus,* high pressure, carbon dioxide, organic chickpea.

## INTRODUCTION

The chickpea, *Cicer arietinum* L. (Fabales: Fabaceae), is one of the most important legume crops in the world. Like other pulse crops, chickpea is traditionally grown as an important crop in Turkey (Erler et al., 2009).

Chickpeas and lentils are not grown extensively in the European Union; therefore EU imports 1.6 million tons pulses with a value of 805 million Euros as per 2007 records. Besides, the quality assurance of organically certified products opened new doors in the European pulses market (CBI Market Survey, 2009). European organic food market has reached 28.7 billion in 2009 with a growth of 12.5% in average between 2005 and 2009 (Donahaye et al., 2007). As per the 2009 data of Agriculture and Rural Affair Ministry of Turkey, 9,433.16 metric tons of organic lentils, 6,306.44 metric tons of organic chickpeas and 777.00 metric tons of beans have been harvested in Turkey (Tarım, 2010).

The seed beetles found in the genus *Callosobruchus* Pic. (Coleoptera: Bruchidae) are economically important pests in stored pulse crops (Erler et al., 2009). It is recorded that 55 to 60% loss in seed weight and 45.50 to 66.30% loss in protein content of pulses is due to infestation caused by these beetles (Islam et al., 2007).

Adult females of *Callosobruchus maculatus* which act as host on various beans and chickpeas lay single fertilized eggs on the external surface of beans. The larva that is hatched from the egg, burrows from the egg through the seed coat into the endosperm of the bean and then undergoes a series of molts and burrows to a position just underneath the seed coat prior to pupation. Although the seed coat of the bean is still intact, about 1 to 2 mm window is apparent at the location where the beetle is pupating to complete the metamorphosis of the larval into a winged adult. After the pupation period has ended, the adult chews through the seed coat and emerges from the bean (Christopher et al., 2009).

As indicated by the Turkish, Europe Union, Japanese, US and Swiss organic regulations and standards, there are strict limitations to the use of chemically synthetic inputs including storage pesticides and fumigants in organic production (Bio Suisse Standards for Production, 2009; Council Regulation, 2007; Organic Foods Production Act of 1990, 2005; Revised JAS Law, 2006). Besides, the chemical control of stored products by insecticides or fumigants presents problems such as

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handling hazards, residues, development of resistance, resurgence and environmental pollution (Shazali et al., 2003). Many chemical fumigants, commercially in use, are considered as carcinogenic and mutagenic agents and they contain carcinogen, mutagen and hepatoxins (Lawrence, 1976). Such concerns and limitation has led to the development of non-chemical methods for the control of insect pests that infest food commodities. One of such methods is the controlled atmosphere (CA), which mainly involves the use of $CO_2$ at atmospheric pressure for grain and pulse fumigation. Although this method is effective, safe and environmentally friendly, it requires very long lethal exposure period to assure complete control. For bruchids, an exposure period of 5 to 6 days is recommended (Shazali et al., 2003).

Several researchers who investigated the use of $CO_2$ as an inert gas at high pressure on different insect pests of cereal grains have reported that the exposure period can be reduced to less than an hour, regardless of the species or the developmental stage. The use of carbon dioxide as a fumigant is accepted by biodynamic and organic markets as it is not considered to be a chemical treatment. High concentrations of $CO_2$ have been shown to be effective in controlling various insect pests (Bendall et al., 2002; George and Sonny, 1998).

The aim of this study is to determine the optimum conditions that can be used to inactivate the adult, pupae, larvae and eggs of *C. maculatus* on chickpeas. The minimum exposure time to pure $CO_2$ (99.90%), sufficient enough to kill *C. macultus* which hosts *Cicer arietinum* under 20 bars at 23 to 26°C, was studied by George and Sonny (1998).

## MATERIALS AND METHODS

In the present study, the adults of *C. maculatus* obtained from 2009 crop Kabuli type organic chickpeas grown in Karkamis town of Gaziantep province, Turkey, were reared in 2009 and 2010. Five liters plastic cups with 190 mm perimeter and 230 mm height were used for the development of stock cultures. The stock culture cups were ventilated through holes with 1 mm perimeter and 1 per $cm^2$. After placing 500 g of organic chickpea samples in each cup, 20 to 25 female and 20 to 25 male *C. maculatus* were transferred to each cup. The organic chickpeas used in the study were all sterilized in the oven for 20 min at 60°C (Iloba et al., 2007; Iloba and Umoetok, 2007). The reason for the sterilization was to kill any egg, larvae or pupae that may likely exist in the chickpeas. The stock cultures were reared at 25 to 32°C and 52 to 76% r.h. After 25 to 35 days of the development period, on the 10th day of adults' emergence, the adults were transferred to new 5 L cups as described earlier, and after the transfer of adults, 500 g of sterile organic chickpeas were added to the stock culture.

The carbon dioxide used in this study was supplied by a company (Habaş, 4099, HB02081621, Istanbul, Turkey) that supplies 99.90% purity carbon dioxide for the food industry in high pressure resistance 45l (25 kg) capacity tubes. Three plastic tubes with 19 mm perimeter and 85 mm height, in addition to a capacity of 9 to 12 chickpeas were used to test adults, chickpeas with pupae, larvae and eggs of *C. maculatus* in the treatment chamber. These tubes were modified for easy gas penetration by opening ventilation holes with 2 mm perimeter amid 5 holes per $cm^2$. The tubes were separately used to test adults and chickpeas with pupae, larvae and eggs. A transferring vehicle with 23 mm perimeter and 85 mm length, plus a jagged top with 35% open surface that was modified from a water pipe, was used to put and easily take out these plastic tubes from the treatment chamber.

To obtain the samples used in the study, cultures in 120 x 20 mm glass Petri dishes were prepared by introducing 10 to 15 adult males and 10 to 15 adult females into 100 to 110 sterile organic chickpeas. Consequently, the cultures were developed in the climatic chamber (Elektormarket, special production, Gaziantep, Turkey) at 30°C and 70% r.h. The adults which were introduced into the cultures were collected back after 5 days to obtain a culture that has the same age or close age in the development stage. When preparing the control and treatment samples, chickpeas with pupae were obtained from 20 to 28 days old culture, while chickpeas with larvae were obtained from 10 to 15 days old cultures and chickpeas with eggs were obtained from 1 to 4 days old cultures. Hatched eggs were determined by a change to creamy colour, resulting from excrete and undigested seed testa particles. However, unhatched eggs are generally transparent, shriveled and wrinkled (Shazali et al., 2003).

Fifty bar pressure resistance and 2 L capacity pig iron tubes (Aytaç, S. no:771, Istanbul, Turkey) with 100 mm perimeter and 350 mm height, plus 30 mm perimeter of the mouth opening were used as the treatment chamber. Moreover, a pressure regulator (Güneş, Istanbul, Turkey) was used to arrange the pressure to be treated. To increase the temperature of $CO_2$ given to the treatment chamber (Alfagama, Felxor, 100 RIAT, Istanbul, Turkey), 2 m length pipes were used between the carbon dioxide stock tube and the treatment chamber. The control and chickpeas samples hosting pupae, larvae and eggs, and also the adults of *C. maculatus* which were tested in the pressure chamber under 20 bars of $CO_2$, were held in climatic chambers at 30°C and 70% r.h. in 60 x 15 mm Petri dishes (Medikal market, ZM-B0069, Istanbul, Turkey), whereas the pupae, larvae and eggs which could not pass the next life stage were considered to be dead. According to the literature, the generation time of *C. maculatus* is 3 to 4 weeks at 30°C, 20 to 40% r.h and 12:12 h day and night, and 5 to 6 weeks at 25°C, 40 to 60% r.h and 12:12 h day and night (Christopher et al., 2009); hence, the samples were monitored for 30 days. During the treatment, $CO_2$ was given to the chamber in 60 to 80 s, while the treatment chamber was emptied in 80 to 90 s.

In the present study, a cross control was also carried simulta neously to determine the effect of high pressure CO2 in bulk appli cations. The high level of infested samples (2 kg) consist of adults, pupae, larvae and eggs of the stock cultures obtained by blending for 1, 4, 11, 15, 28, 34 and 44 days, and which were tested at 20 bars in different exposure times with fifty grams control samples taken before the treatment of each 1.5 kg samples, and another 50 g sample taken after the treatment was kept in climatic chambers at 30°C, 70% r.h. and 12:12 day and night period in separate 120 x 20 mm Petri dishes for 30 days (Reichmuth and Ofuya, 2001).

The remaining parts of the treated samples were taken into 5 L plastic cups, described earlier and monitored for 6 weeks under 25 to 32°C, 52 to 76% r.h. and day light conditions.

## RESULTS AND DISCUSSION

In the present study, with regards to the tests that were done by using 9 to 12 chickpeas capacity plastic tubes, 100% mortality was determined in 50 and 90 min at 26°C and 20 bars with chickpeas carrying eggs, and at 24°C and 20 bars with chickpeas bearing larvae, while 100%

**Table 1.** Mortality in adults, pupae, larvae and eggs of *C. maculates* hosting on chickpeas in different exposure times exposed to 99.90% purity $CO_2$ at 20 bar pressure.

| Exposure time (min) | Exposure temperature (°C) | Adult | Pupae (%) | Larvae (%) | Egg (%) |
|---|---|---|---|---|---|
| 240 | 24 | 100% | 100.00 | 100.00 | 100.00 |
| Mortality in control group | | N.A. | 11.11 | 13.37 | 52.95 |
| 180 | 24 | 100% | 100.00 | 100.00 | 100.00 |
| Mortality in control group | | N.A. | 0.00 | 52.63 | 5.88 |
| 150 | 26 | 100% | 100.00 | 100.00 | 100.00 |
| Mortality in control group | | N.A. | 0.00 | 35.00 | 00.00 |
| 120 | 25 | 100% | *100.00 | 100.00 | 100.00 |
| Mortality in control group | | N.A. | 8.87 | 18.57 | 14.29 |
| 90 | 24 | 100% | 95.00 | *100.00 | 100.00 |
| Mortality in control group | | N.A. | 0.00 | 35.49 | 34.18 |
| 60 | 24 | 100% | 81.88 | 88.89 | 100.00 |
| Mortality in control group | | N.A. | 0.00 | 50.00 | 11.11 |
| 50 | 26 | 100% | 60.00 | 30.00 | *100.00 |
| Mortality in control group | | N.A. | 0.00 | 0.00 | 31.58 |
| 40 | 26 | 100% | 75.00 | 70.00 | 88.89 |
| Mortality in control group | | N.A. | 0.00 | 10.53 | 20.00 |
| 30 | 24 | 100% | 0.00 | 22.22 | 90.00 |
| Mortality in control group | | N.A. | 0.00 | 0.00 | 5.00 |
| 20 | 24 | 100% | 58.33 | 60.00 | 90.00 |
| Mortality in control group | | N.A. | 0.00 | 0.00 | 10.00 |
| 10 | 25 | 100% | 36.36 | 55.55 | 62.50 |
| Mortality in control group | | N.A. | 0.00 | 12.50 | 0.00 |
| 5 | 23 | 100% | 0.00 | 50.00 | 45.45 |
| Mortality in control group | | N.A. | 0.00 | 20.00 | 5.00 |
| 1 | 22 | 100% | 0.00 | 0.00 | 22.22 |
| Mortality in control group | | N.A. | 0.00 | 0.00 | 26.08 |

*100% mortality at minimum exposure time.

mortality in chickpeas with pupae was achieved only after 120 min exposure time. In the test, it was also determined that 1 min treatment at 24°C and 20 bars was sufficient to kill all the adult males and females of *C. macultus* (Table 1).

In the cross control study which was carried at 22 to 26°C and 20 bars by loading the highly infested chickpeas in bulk in the treatment chamber, the survival continued after 1, 10, 20, 40, 60 and 120 min application, while no development was monitored after 180 and 240 min exposure times (Table 2).

*C. maculatus* is an insect that is easy to rear and maintain and it has a very rapid life cycle. At 25 to 30°C, pupation and emergence of an adult beetle occurs in 25 to 35 days after an egg is deposited. Adults become mature in 24 to 36 h after emergence and they do not need to be fed. Under these circumstances, adults may live for an average period of 12 to 14 days during which time, mating and oviposition occurs. Adult sexes can be distinguished by means of morphological differences that are easily seen. Females have dark stripes, which are not found in males, on each side of the posterior dorsal abdomen. Nevertheless, adults have an average body length of 4 to 6 mm (Christopher et al., 2009).

The lethal action of $CO_2$ is related to the increased solubility of $CO_2$ in the insect body fluids under high pressure, causing a subsequent decrease in pH. It has also been reported that a dramatic increase in the uptake of $CO_2$ under high pressure causes rapid expansion and rapid evaporation from the liquid when the pressure is reduced, resulting in lesions in the cell membranes of adult and larva. The integument of insects exposed to the treatment was severely damaged due to the expansion of internally dissolved $CO_2$ in the body when the gas pressure was rapidly reduced to atmospheric pressure. While 35 days is needed for 100% mortality of *Sitaphilus granairus L.* eggs which are known with their high tolerances under atmospheric pressure at 10°C and 90% $CO_2$ concentration, and under 20 bars pressure at 10°C and 99% $CO_2$ concentration, the treatment time dramatically decreased to 3 h (Nielsen, 2001). The effect of $CO_2$ also depends on the stage of development, the level of

**Table 2.** Development of *C. maculates* in highly infested chickpeas after exposure to 99.90% purity $CO_2$ at 20 bar pressure in different exposure times.

| Exposure time (min) | Exposure temperature (°C) | Treated chickpeas samples held in room conditions (25 to 30°C and 50 to 70% r.h.) | Treated chickpeas samples held in the climatic chamber (30°C and 70% r.h.) | Survival success in control groups |
|---|---|---|---|---|
| 240 | 26 | - | - | + |
| 180 | 26 | - | *- | + |
| 120 | 24 | *- | + | + |
| 90 | 26 | + | + | + |
| 60 | 25 | + | + | + |
| 40 | 26 | + | + | + |
| 20 | 25 | + | + | + |
| 10 | 25 | + | + | + |
| 1 | 25 | + | + | + |

*100% mortality at minimum exposure time.

pressure and the time of exposure (Shazali et al., 2003). Besides, $CO_2$ is known to inhibit respiration (Nielsen, 2001). This is probably due to low levels of oxygen and high carbon dioxide, which hinders the oxidative breakdown of pyruvate that is required for energy release from food.

As per the results of both tests, it was determined that at least 3 h treatment at 22 to 26°C and 20 bars is required to kill the entire development stage of *C. maculates* on organic Kabuli type of Turkish origin chickpeas with 99.90% purity of carbon dioxide. In the present study, it is also noted that the pupae stage of *C. maculates* has higher tolerance against high pressure carbon dioxide.

The data obtained in the present study contradict with the results of the study carried out by Shazali et al. (2003) which proved that $CO_2$ treatment for 20 min is adequate to kill the 4 to 5 days old eggs at 20 bars, but the findings support the results of the studies done by Gerard et al. (1988) on drugstore beetle, *Stegobium paniceum*, which show that 100% mortality, in the entire developmental stages, is obtained at 20 bar in 2 h (Nielsen, 2001).

Contrary to the other studies that have been done on other insect species, which report that the eggs are more tolerant in the developmental stage against $CO_2$ treatments, in the present study, it is found that the pupae and larvae of *C. maculatus* are more tolerant to high pressure $CO_2$ than the eggs.

It is reported that the time loading of $CO_2$ needed to obtain the requested pressure and the time required to empty the tank and reduce the pressure to atmospheric conditions have effects on the mortality of insects (Shazali et al., 2003). Therefore, it is necessary for studies to be done on the effect of this condition on the mortality of insects under high pressure $CO_2$.

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