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Studies on respiratory toxicity of Acetone and Carbon dioxide on red flour beetle *Tribolium castaneum* (Herbst)

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Abstract
In this research, two environmentally compatible compounds namely Acetone and Carbon dioxide were evaluated for their efficacy against of *Tribolium castaneum* (Herbst), under laboratory conditions. The LC_{25} and LC_{50} values of Acetone and Carbon dioxide were estimated on adult insects. Furthermore, LC_{25} and LC_{50} values for Acetone and Carbon dioxide treatments on adult insect were 9.84 and 23.24 $\mu l\ L^{-1}$, 11.08 and 19.21 mg L^{-1}, respectively. After determining the LC_{50} and LC_{25} values of two agents, to evaluate the combined effects of Acetone and Carbon dioxide an experiment was conducted in the form of completely randomized design with four treatments including LC_{50} (Ac), LC_{50} (Co_2), LC_{25} (Ac) plus LC_{25} (Co_2), and control. Our results demonstrated that Carbon dioxide was more effective. The results showed that percentage mortality combination two environmentally compatible compounds treatments on adult insect in 24, 48 and 72 h were 36.6, 66.6 and 90 percent compared with Acetone (16.6, 33.33 and 50) and Carbon dioxide (20, 36.6 and 53.33) alone and control have significant differences ($P<0.05$).The results showed that the combination of Acetone and Carbon dioxide have good additive effect when compared with applied individually treatments against of one important stored product pest.

Keywords: *Tribolium castaneum*, Acetone, carbon dioxide, LC_{25}, LC_{50}

Introduction

Stored products with herbal and animal source are attacked by 600 species of Coleoptera, 70 species of Lepidoptera and 355 species of mites and cause many quantitative and qualitative damages [19]. Speed of population growth of storehouse pests like Coleoptera and Lepidoptera is very high and consequently amount of caused damage is very high. Kind of stored agricultural products is very effective in pest population growth, because pest quality and quantity can have significant effect in biologic activities and consequently has population density [4]. Storehouse pest insects in storehouse ambient because of good biologic situation can act whole the year. According to above cases, to reduce the damages, pest population control is inevitable and in this cases using of chemical pesticides is common, it had good effects at first but these results were very unstable because in a short time after using these pesticides many problems were appeared that for example we can name pest resistance, pesticides aggregation in human body, causing many diseases because of remained pesticide on products and increase in cost of pesticides application [16]. In recent years, cereals, especially wheat, rice, malt, and corn has made human's main food, so storehouse building and storage of products for human societies is very important from past times. Annually more than hundred tons of cereals because of damages of storehouse pests and nonconformity of scientific basics of storage are wasted that because of lack of progress and complement of scientific methods this amount of damage is more in undeveloped countries than developed countries [11]. Numerous investigators have studied the application and effectiveness of fumigants to control stored-product insects [18]. Fumigants are widely used for the disinfecting of commodities and treatment of empty stores. In the last years the removal of some fumigants from the market has resulted in a wider use of methyl bromide and phosphine [12]. In recent years, cereals, especially wheat, rice, malt, and corn has made human's main food, so storehouse building and storage of products for human societies is very important from past times. Annually more than hundred tons of cereals because of damages of storehouse pests and nonconformity of scientific basics of storage are wasted that because of lack of progress and complement of scientific methods this amount of damage is more in undeveloped countries than developed countries [11].

Acetone is generally recognized as a less deleterious substance to man and the environment, inexpensive, commonly available and convenient to use [20]. Acetone is widely used in laboratories as a solvent in applying insecticides to insects. It is absorbed through the skin, but lungs and kidneys excrete considerable amounts of absorbed Acetone in a short period of time [8]. The treatment meets the demands of the organic market. The technology can be adopted where cheap sources of CO_2 are available and the storage structure is well sealed. CO_2-rich atmosphere has been found suitable for the protection of dried fruits in Turkey [7, 9] and for treating grain elevators in Canada [14]. CO_2 treatment under elevated temperatures and with low concentrations of phosphine has been recommended as a replacement for methyl bromide in treating flour mills and other food processing facilities in Canada and other developed countries [17]. CO_2 is not carcinogenic and has no adverse effects on the environment. Carbon dioxide treatment requires a long period of 10 days or more. This drawback can be overcome by raising the treatment temperature or by applying the gas under high pressure. Carbon dioxide up to 30% atmospheric composition is tolerable to insects [13, 15].

In present study, respiratory toxicity of Acetone and Carbon dioxide on *Tribolium castaneum* was evaluated.

Material and Methods

The present study was carried out in entomology lab of agriculture faculty, plant protection department of Urmia University at summer of 2015 in Iran.

Insect rearing

The red flour beetle, *T. castaneum* was used for the present experiments. A colony of *T. castaneum* was obtained from storage products in the laboratory of Urmia University. They were reared under storage conditions, on flour at 27±2 °C, with L: D 14: 10 and 65±5% RH.

Insecticides tested

In this study, the test Acetone was 99.9% and supplied by Merck Co. Ltd. This compound is polar and a highly volatile and flammable liquid (Howard, 1991) [10]. The carbon dioxide gas was applied to containers from a vessel of liquid carbon dioxide with appropriate vaporizers and pressure regulators to control the flow rate. To determine the LC_{50} values of carbon dioxide mixture with Acetone, the procedure developed by White and Collins [5] was adopted. The test containers each of 31 L capacity containing wheat and insects were used in these tests. The samples were exposed to different dosages of CO_2 at 27±2 °C. Due to on field and applied nature of the research the volume of carbon dioxide in the chamber air was overlooked. Preliminary tests revealed that the atmospheric composition containing ≈ 10% CO_2 is harmless to insects. The atmospheric composition of chamber was modified to contain 10% CO_2 and LC_{50} dosage of Acetone was introduced to the chamber. The test containers and control group were stored at 27±2 °C for 24 h.

Determine of LC_{50} and LC_{25}

To estimate the LC_{50} and LC_{25} five concentrations from each insecticide after primary experiments with distill air as control treatment in three replicates were used in cast iron capsules (51 L) on 10 adult pests in petri dish and mortality was recorded after 24 hours.

The interaction effects between Acetone and CO_2

After calculating LC_{50} and LC_{25} values for Acetone and CO_2 on adult stages, combination effects of Acetone and CO_2 in petri dish in capsules were evaluated. All experiments in completely randomized design in 4 treatments include LC_{50} of Acetone, LC_{50} of CO_2, LC_{25} Acetone plus LC_{25} CO_2 and distilled air control in three replicates after drying, filter papers were put in petri dishes and 10 of adult *T. castaneum* was selected and added on petri dishes and were covered completely with parafilm glue. After 24, 48 and 72 hours percentage of mortality was recorded.

Analysis of Data

The LC_{50} and LC_{25} values (with 95% confidence limits) were calculated by using Probit Analysis Statistical Method, mortality data treatments subjected to analysis of variance (One way ANOVA) and mean separation tests were conducted with Tukey's HSD with SPSS statistical analysis software (Ver. 22.0).

Results

LC_{50} and LC_{25} Acetone and CO_2 on adult stage:

LC_{50} and LC_{25} of Acetone and CO_2 on adult stages are shown in Table 1.

Table 1: LC_{50} and LC_{25} values Acetone and CO_2 effect on adult red flour beetle within 24 hours

Insecticide	Time (hours)	Slope±SE	Chi-square	Lethal concentration	
				LC_{25} 95% confidence interval	LC_{50} 95% confidence interval
Acetone	24	-2. 50±1.82	4.62	9.84 (7.78-12.12)	23.24 (20.17-27.41)
CO_2	24	-3.67±2.85	6.71	11.08 (5.35-14.74)	19.21 (14.82-25.53)

Interaction effects of Acetone and CO_2 on *T. castaneum* in 24 H

Effects of treatments, Acetone LC_{50}, CO_2 LC_{50} and Acetone plus CO_2 on *T. castaneum* was evaluated and counting the percentage mortality after 24 H (fig. 1). The results showed that there was a significant difference between LC_{25} Acetone+LC_{25} CO2 treatment with alone application treatments LC_{50} Acetone andLC_{50} CO_2 with 99% confidence in 24 H [$F(3,11) = 40.667$, p=0.001)]. According to result showed that combined effects of treatments the highest mortality compared with other treatments.

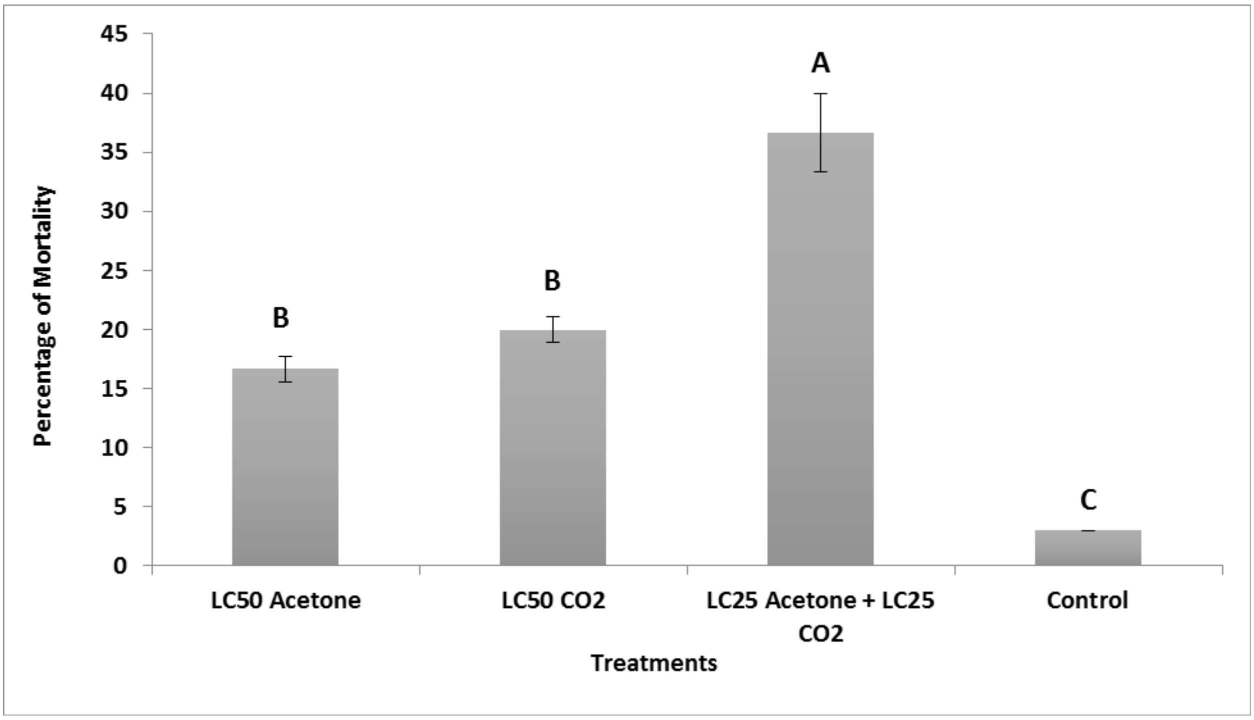

Fig 1: Interaction effects of Acetone and CO_2 on *T. castaneum* in 24 H

Interaction effects of Acetone and CO_2 on *T. castaneum* in 48 H

Effects of treatments, Acetone LC_{50}, CO_2 LC_{50} and Acetone plus CO_2 on *T. castaneum* was evaluated and counting the percentage mortality after 48H (fig. 2). The results showed that there was a significant difference between LC_{25} Acetone+LC_{25} CO_2 treatment with alone application treatments LC_{50} Acetone andLC_{50} CO_2 with 99% confidence in 48 H [$F(3,11) = 34.476$, p=0.001)]. According to result showed that combined effects of treatments the highest mortality compared with other treatments.

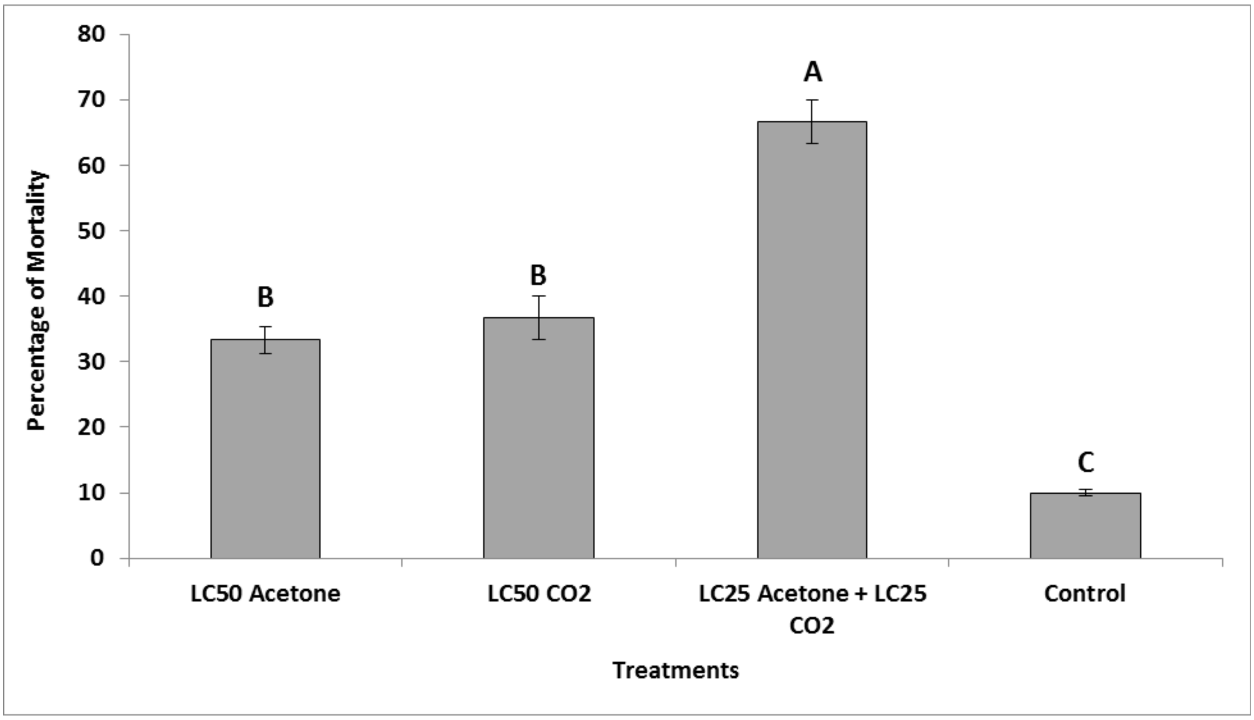

Fig 2: Interaction effects of Acetone and CO_2 on *T. castaneum* in 48 H

Interaction effects of Acetone and CO_2 on *T. castaneum* in 72

Effects of treatments, Acetone LC_{50}, CO_2 LC_{50} and Acetone plus CO_2 on *T. castaneum* was evaluated and counting the percentage mortality after 72H (fig. 3). The results showed that there was a significant difference between LC_{25} Acetone + LC_{25} CO2 treatment with alone application treatments LC_{50} Acetone andLC_{50} CO_2 with 99% confidence in 72 H [$F (3, 11) = 87.733$, p=0.001)]. According to result showed that combined effects of treatments the highest mortality compared with other treatments.

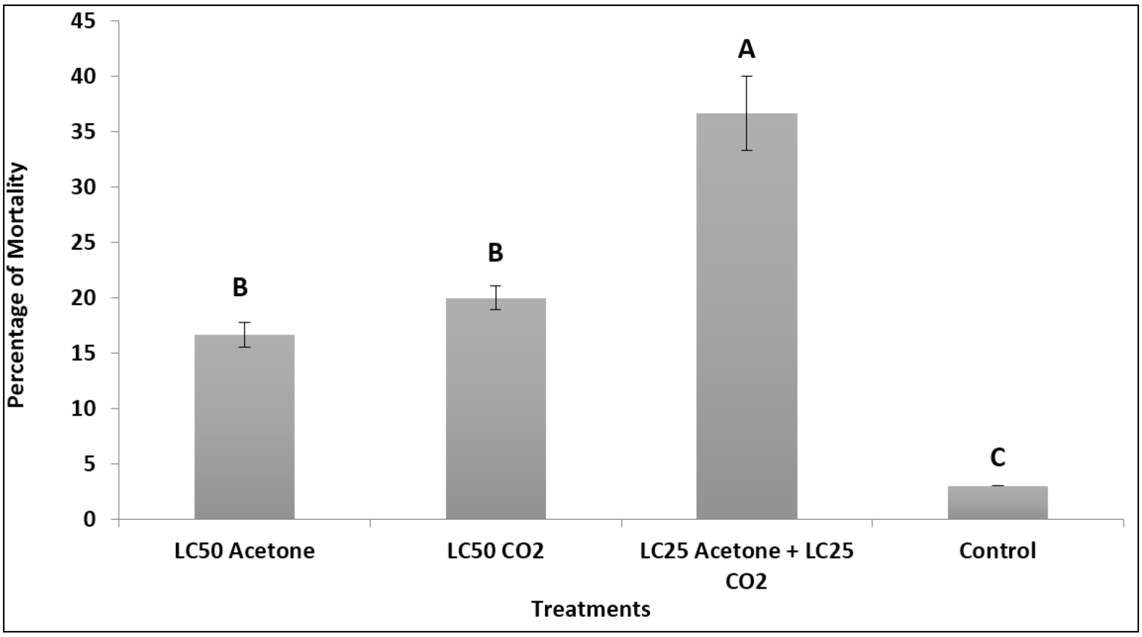

Fig 3: Interaction effects of Acetone and CO_2 on *T. castaneum* in 72 H

Discussion

For the control of stored-products pest insects, particularly in grain, farmers rely mostly on the treatment of contact insecticide to raw cereals [2, 6]. Because such treatments may result in the presence of residues in those products, there are restrictions in the level of insecticide residues allowed in such products [2, 3]. Therefore, the number of suitable contact insecticides that can be used in the control of stored-products insects is limited [22, 1]. For a long time, the main stored-grain protectant insecticide was de-odorized malathion [6, 1]. Unfortunately, most stored-products insects are substantially resistant to this insecticide and an alternative substitution should be necessary [12, 1]. Fumigation is one of the most successful methods of rapidly controlling insects infesting stored foodstuffs. The cost and health risk of fumigation seems to be lower than traditional methods of preservation [21]. Thus, it appears that fumigation will be the backbone and indispensable component of stored-products insects control programs in the immediate future. A good fumigant should have some characteristics consistent with the fumigation protocol, which ensures an appropriate level of insect control and produces the minimum of hazardous side effects [2]. Unfortunately, the two available fumigants fall short of this ideal. At the present time, large proportions of stored foodstuffs are fumigated with methyl bromide and phosphine. The greatest deficiency in the use of methyl bromide was that in many instances the major reliance has been placed on the methyl bromide fumigation and the stock management was neglected. Therefore, reinfestation occurred soon after the fumigation was completed. Consequently, frequent fumigation was necessary and grain often had bromide residues in excess of the permissible level. Phosphine as a fumigant offers a cost-effective method of insects control [18]. Strict controls on detectable concentrations of phosphine are necessarily imposed by some organizations. Since excessive residue from fumigation is a potential hazard to consumers, methyl bromide and phosphine are under close scrutiny and will have limited use in the immediate future [21]. Acetone is absorbed through the skin and is distributed throughout the body. The fatal dose of Acetone for an average adult lies between 300 and 400 mL, if this amount is ingested in less than an hour [8]. Therefore, death from Acetone should be extremely uncommon under fumigation conditions. Although Acetone is not a novel compound, as yet it is not registered for use as a fumigant. However, in view of the wide usage of Acetone in toxicological studies, information on its action against insects including as a fumigant could be useful in interpretation of toxicological data. In the current study, Acetone was toxic to all tested insects in empty-space tests. This finding would agree with the data collected by Tunç *et al*., that Acetone is generally recognized as a less deleterious substance to man and the environment, inexpensive, commonly available and convenient to use [20].

The mixture of carbon dioxide with CO_2 can be considered as a potential fumigant for replacing methyl bromide or phosphine under ambient storage conditions specifically in empty-space fumigations

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