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Studies on Respiratory Toxicity of Acetone and Carbon dioxide on third instar larva *Plodia interpunctella* (Hubner)

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ABSTRACT: Control Storehouse pest insects is the main objectives of agricultural products, manufacturers, exporters and consumers by maintaining the quality and quantity of products around the world. In this research, two environmentally compatible compounds namely Acetone and Carbon dioxide were evaluated for their efficacy in the control of *Plodia interpunctella*, under laboratory conditions. LC25 and LC50 values for Acetone and Carbon dioxide treatments on third instar larva insect were 56.32 and 68.31 μlL^{-1}, 7.78 and 15.38 mgL^{-1} respectively. To evaluate the combined effects an experiment was conducted in the form of completely randomized design with 4 treatments including LC50 (Ac), LC50 (CO_2), LC25 (Ac) plus LC25 (CO_2), and control. The results showed that percentage mortality combination two environmentally compatible compounds treatments on third instar larva insect in 24h was 75 percent compared with Acetone and Carbon dioxide alone and control have significant differences And good additive effect ($P<0.05$).

Keywords: *Plodia interpunctella*, Acetone, carbon dioxide, LC25, LC50

INTRODUCTION

According to statics in average 35% of agricultural products are waste that this amount of product can supply 15 to 20 million people's food. According to Climate diversity, many herbal species and other abilities in agricultural goods production, has very high capacity of export in agricultural whereas agriculture is economical support for countries and can create many job opportunities (FAO, 2009). Damages of storehouse pest in developed countries is 9 percent and in developing countries is more than 20% (Phillips and Thoren, 2010). On average, Storehouse pest, cause losses of 10% cereals in around the world (Carvalho-Barbosa-Negrisoli *et al.*, 2013). Storehouse pest insects, reducing the quality of food and health abnormalities, therefore international organizations, such as FAO (2009) and FGIS (1999) have announced insects in the foods for human consumption is unacceptable.

Damaged caused by insects, mites, fungus, and seed's germination in storehouse cause many wastes to stored products (Harein and Meronuck, 1995). 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 (Rajendran, 2002). Storehouse pest insects in storehouse ambient because of good biologic situation can act whole the year. 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 (Cassanova, 2002) 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 (Mason and Strait, 1998). 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 (Jayas *et al.,* 2006). Numerous investigators have studied the application and effectiveness of fumigants to control stored-product insects (Rajendran and Muralidharan, 2001). 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 (Leesch, 1995).

Acetone is generally recognized as a less deleterious substance to man and the environment, inexpensive, commonly available and convenient to use (Tunc *et al.*, 1997). 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 (Gossel and Bricker, 1990). Treatment of stored food commodities with CO_2-riched atmospheres has been studied previously (MBTOC, 1998). 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 (Donahaye *et al.*, 1998; Ferizli and Emekci, 2000) and for treating grain elevators in Canada (Macrotte and Tibelius, 1998). Combined effects of CO_2 with low concentrations of phosphine has been recommended as a replacement for methyl bromide in treating Stored-product Insect Including *P. interpunctata* (Valizadegan *et al.*, 2012). If carbon dioxide at a concentration of 35% alone use in food storage, fumigations with water absorption cell tissue will reduce triglycerides insects, thus disrupt the body's metabolism causing the death of its insecticides (Leelaja *et al.*, 2006). 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 (Leoge *et al*., 1995; Manna *et al.*, 1999).Control Storehouse pest insects is the main objectives of agricultural products, manufacturers, exporters and consumers by maintaining the quality and quantity of products around the world.

In this context, to improve the quality and quantity of fumigations and providing sound solutions to existing facilities, this study was conducted. In present study, respiratory toxicity of Acetone and Carbon dioxide on of *Plodia interpunctella* (Hubner) was evaluated.

MATERIAL AND METHODS

The present study was carried out in entomology lab of agriculture faculty, plant protection departments of Urmia University of 2013-2014 in Iran.

A. Insect rearing

Indian meal moth, *Plodia interpunctella* was used for the present experiments. A colony of *P. interpunctella* was obtained from storage production the laboratory of Urmia University. Reared Indian meal moth on artificial diets recommended by Sait *et al* (1997) contains a mixture of 800 (g) of wheat bran, yeast (160 g), glycerol 200 cubic centimeters and 200 grams of honeyand at 28±2 °C, with L: D 13: 11 and 65±5% RH.

B. 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). 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. The test containers each of 51L capacity containing on artificial diets and insects were used in these tests. The samples were exposed to different dosages of CO_2 at 26±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 LD50 dosage of acetone was introduced to the chamber. The test containers and control group were stored at 28±2°C for 24 h.

Test of LC50 and LC25. To estimate the LC50 and LC25 five concentration from each insecticides after primary experiments with distill air as control treatment in three replication used in Cast iron Capsules (51 L) on 10 third instar larva pests in petridish and mortality recorded after 24 hours .

C. The interaction effects between Acetone and CO_2

After calculating LC50 and LC25 values for Acetone and CO_2 on third instar larva 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 LC50 of Acetone, LC50 of CO_2, LC25 Acetone plus LC25 CO_2 and distilled air control in three replications After drying, filter papers were put in petri dishes and 10 of third instar larva *P. interpunctella* was selected and added and petri dishes were covered completely with parafilm glue. After 24 hours percentage of mortality was recorded.

D. Analysis of Data

The LC50 and LC25 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 (Version 22.0).

RESULTS AND DISCUSSION

Bioassay:

LC_{50} and LC_{25} Acetone and CO2 on third instar larva stage:

LC50 and LC25 of Acetone and CO2 on third instar larva **stages** in times 24 h are shown in Table 1.

Interaction effects of Acetone and CO_2 on *P. interpunctella* in 24 H:

Effects of treatments, Aceton LC50, CO_2 LC50 and Acetone plus CO_2 on *P. interpunctella* was evaluated and counting the percentage mortality after 24H (Fig. 1). The results showed that there was a significant difference between LC25 Acetone+LC25 CO_2 treatment with alone application treatments LC50 Acetone and LC50 CO_2 with 99% confidence in 24 H [$F(4, 10) = 19.15$, $p=0.001$)]. The results indicate a synergistic effect that causes 75% mortality in the test mixture. According to result showed that combined effects of treatments the highest mortality compared with other treatments.

Table 1: LC50 and LC25 Values Acetone and CO_2 effect on third instar larva within 24 hours.

Insecticide	Time (hours)	Slope±SE	Chi-square	Lethal concentration (…)	
				LC_{25} 95% confidence interval	LC_{50} 95% confidence interval
Acetone	24	8.04±3.08	0.12	56.32 (49.25-61.87)	68.31 (63.19-76.02)
CO_2	24	2. 28±0.46	2.06	7.78 (4.01-11.24)	15.38 (12.72-18.33)

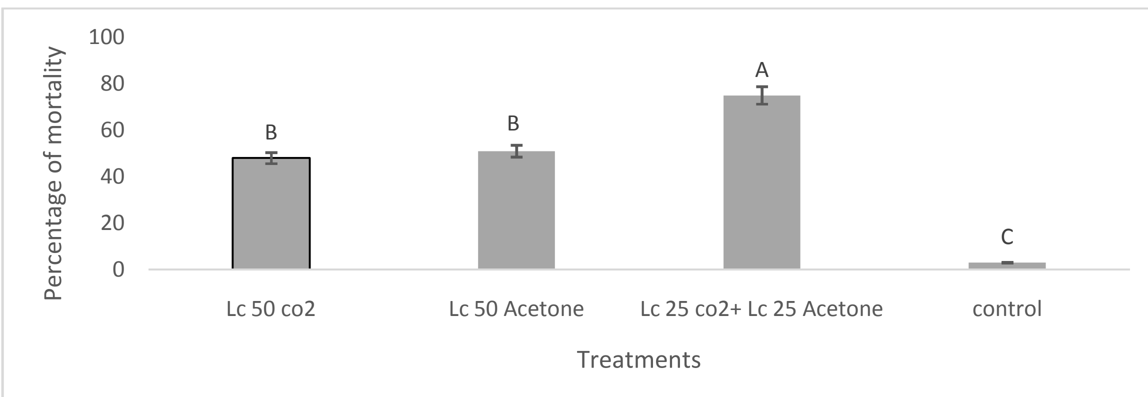

Fig. 1. Interaction effects of Acetone and CO_2 on *P. interpunctella* in 24 H.

Under practical conditions, there isn't any toxic fumigant harmless and there is no little danger to humans and the environment. The fumigations each has shortcomings that limited the use of lead, so the list is very long fumigating in the past two decades. Only a few remained and new alternatives must be based on technology that involves the use of simple and effective methods (Navarro, 2006).

For the control of stored-products pest insects, particularly in grain, farmers rely mostly on the treatment of contact insecticide to raw cereals (Bond, 1984; Daglish, 1998). Because such treatments may result in the presence of residues in those products prepared from treated grain, there are restrictions in the level of insecticide residues allowed in such products (Bond, 1984; Brewer *et al.*, 1994).

Therefore, the number of suitable contact insecticides that can be used in the control of stored-products insects are limited (White and Leesch, 1996; Arthur, 1999). 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 (Weller and Morton, 2001). Based on Valizadegan *et al.*, (2012), LC50 of carbon dioxide on larval stage of *Plodia interpunctella* and *Sitotroga cerealella* as adult stage of *Lasioderma serricorne* and *Oryzaephilus surinamensis* were 47.5, 13.6, 10.12 and 67.7 ppm, respectively which have relative lethal effect in lower than recommended doses. These results were compatible with our study.

Sauer and Shelton (2000) evaluated 60% concentration effect of carbon dioxide in 18 and 24 hours fumigation on *Plodia interpunctella* and reported that some cases led to 100% mortality which this survey was similar to our results. This result was not far from of expectation, because with increasing of fumigation period times, the insects expose to more toxin. According to tajbakhsh and Pourmirza (2008), aceton is harmless to viability of wheat seed and caused to relative control pests. According to LC50 of carbon dioxide on three species of stored product pests, the highest mortality was related to *Plodia interpunctella* and the lowest mortality was related to *Callosobruchus maculates*. In the other hand use of carbon dioxide combined with other fumigants improved fumigation results by increasing of insect respiration. These results compatible with (Prozell *et al.*, 1997).

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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