INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
21–0871/2022/27–1–77–82
DOI: 10.17957/IJAB/15.1901
http://www.fspublishers.org

Full Length Article

Modified Atmosphere as an Alternative Measure for Controlling of *Sitophilus oryzae* Reared on Different Stored Grains

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Received 07 September 2021; Accepted 28 December 2021; Published 30 January 2022

Abstract

Protection of grains and their products from insect pests remained a big constraint in the way of food security. Current study evaluated the effective exposure time to kill the *Sitophilus oryzae* (L.), reared on two different diets *viz.*, wheat and maize under six Carbon dioxide (CO_2) concentrations. Modified atmospheres (MA) contained 25, 30, 35, 40, 45 and 50% CO_2 by volume at ambient temperature, respectively. Twenty *S. oryzae* adults, along with a 20 g diet, were released in each airtight exposure chamber (150 mL capacity). A measured quantity of CO_2 gas (99.9%) was released in exposure chamber by the injection syringe from gas cylinder. Mortality data were recorded after 24, 48, 72, 96 and 120 h. The mortality rates varied between the insect cultures reared on maize and wheat diets. *S. oryzae*, reared on a wheat diet, showed higher mortality after exposure to all CO_2 concentrations as compared to the insect culture reared on the maize diet. At 45% CO_2concentration, maximum mortality (100%) was observed after 120 h in case of maize reared insects and (100%) after 96 h in case of wheat reared *S. oryzae*. The results revealed that Carbon dioxide, as an eco-friendly approach, may be used as the best alternative method to minimize the pest infestation in stored products to avoid insecticide resistance development in stored grain insect pests. The Carbon dioxide is not included in the category of toxic gases and has no detrimental or residual effect in the stored grains. © 2022 Friends Science Publishers

Keywords: Carbon dioxide; Modified atmosphere; *Sitophilus oryzae*; Mortality response

Introduction

Stored grains are a very important source of calories and proteins for human throughout the world but arthropod pests play a negative role by disturbing the supply of these grains. Insect pests damaged grains have low marketing value because of less nutritive quality and bad taste (Alonso-Amelot and Avila-Núñez 2011). Insect pests of stored grains damage stored products by causing 5–10% losses globally while 10–40% losses in developing countries (Weaver and Petroff 2005), including Pakistan (Ahmad 1984). Human survival mainly depends upon the wheat crop because it contributes major part in the economy of any country (Yu *et al.* 2017). Maize is also main and important food of the world (Kennett *et al.* 2020). In agriculture, grains constitute the major component of food items. Grains like rice, wheat and maize cover 43% food calories and 87% grain production, globally. To fulfill the food requirement of 42% population of the world, we depend upon maize (Khan 2018).

Unfortunately, these grains are attacked by numerous kinds of insect pests during storage conditions. Coleopteron and Lepidopteron insect pests are the most significant insect pests causing huge destruction to grains in the field and storage (Emana and Tsedeke 1999). The coleopteron insects attacking stored grains during storage are four types' beetles. Among these beetles, rice weevil (*Sitophilus oryzae* L.) is widely distributed globally. It is very destructive primary pest of stored grain, mostly prefer soft grain varieties (Pathak and Jha 2003; Padmasri *et al.* 2017).

Insect pests of stored grains and their products have been controlled by the application of insecticides. Due to repetition of insecticides application, insect pests have developed insecticide resistance against these insecticides (Wallbank and Collins 2003). Besides the development of insecticide resistance in insects, some of these chemicals are also banned due to hazardous effects on human health (Isman 2006) and the environment (Haines 1995). Resistance and harmfulness issues of synthetic insecticides

To cite this paper: Mohsan M, ZM Sarwar, Z Hussain, MS Asghar, MA Badar, MR Khan (2022). Modified atmosphere as an alternative measure for controlling of *Sitophilus oryzae* (L.) reared on different stored grains. *Intl J Agric Biol* 27:77–82

have brought about the needs of discovering more viable and healthier alternatives.

It is in high demand of the food industry to control arthropod pests through different eco-friendly control methods. These techniques are also promoted by the government through financial support and legislation because the food market needs increased demand for organic grains. Therefore, different non-chemical and eco-friendly methods have been adopted. Among these, the modified atmosphere (MA) approach is suitable for the control of stored product insect pests (Adler *et al.* 2000; Navarro 2006). To overcome the infestation of stored commodities pests, the modified atmosphere technique is considered an environmentally safe method. Montreal protocol, an international agreement, suggested the scientists develop different alternatives tore place methyl bromide and similar products due to health and environmental hazards (Fields and White 2002).

The use of CO_2 as an eco-friendly approach is one of the best options to control stored insect pests, because this approach has no harmful effects on stored food (Husain *et al.* 2017). This gas adversely affects the functions of normal body of beetles a including system of hormones, nervous system, digestive system, and circulatory system (Nicolas and Sillans 1989) because gas particles enter the insect body and open the spiracles permanently (Jay *et al.* 1971). Carbon dioxide can absorb and desorbed in grains without any chemical reaction and pose almost no effect on grains chemistry (Yamamoto *et al.* 1980). Management strategies to control all the developmental stages of pests through a modified atmosphere take several weeks because it depends upon the gas concentrations (Riudavets *et al.* 2009).

The 1st objective of the research was to assess the required exposure time to get 100% mortality by exposing the adult stage of *S. oryzae* to CO_2 at 27 ± 2°C temperature and 65 ± 5% relative humidity (RH). The 2nd objective of this research was to deliberate the notions and variations of MA, its impact on pests and on the superiority of the product being treated, the structure where it may be considered for use, and its compatibility in commercial settings.

Materials and Methods

Rearing of homogenous insect culture

The population of *S. oryzae* was collected from stored grains of the local grain market of district Multan. The collected population was reared under optimum laboratory conditions at 27 ± 2°C and 65% Relative humidity. Insect cultures were reared separately on two diets *i.e.*, wheat and maize in sterilized ventilated plastic jars (1 L). Grains were also sterilized using an incubator at 50°C for 10 minutes to kill all living entities if present in grains. Moisture of grain was maintained at optimal conditions (27 ± 2°C and 65%) for rearing *S. oryzae*. From both cultures, hundred adult pairs were released in separate plastic jars with fresh diets for egg laying to produce a homogeneous F_1 generation. After 2–3 days, adults were sieved out and eggs were allowed behind on the diets for hatching. These larvae were provided optimum rearing conditions to pupate. Pupae of first day were collected in separate bottles on regular basis to obtain a homogenized population. Three days old adults of these homogenized populations were used for the experiment (Sarwar *et al.* 2020).

Carbon dioxide (CO_2) source

Carbon dioxide (CO_2) 99.9% gas cylinder was obtained from medical gas supplier company, Faisalabad Punjab Pakistan.

Gas purity analysis

Biogas analyzer BIOGAS 5000 manufactured by "Geotech" was used to confirm the purity of CO_2 gas.

Gas application

Sterilized transparent plastic bottles (250 mL) were used as exposure chambers. Each bottle was filled with 20 g sterilized respective diet to release 20 adult insects before injecting gas. Bottles were tightly plugged with special rubber septa. CO_2 gas was applied by injection syringe. The injection syringe was connected to a three ways stopper to control the movement of gas, one side was connected to a short hose coming from gas cylinder, other was connected to the needle for injecting gas and third one was used to regulate the CO_2 gas. A measured quantity of gas was injected into the exposure chamber after evacuating the same volume from exposure chamber through the injection plunger. Cylinder pressure was controlled by using pressure regulator gauges. After treatment bottles were kept undisturbed for definite periods inside the incubator at 27 ± 2°C and 65% R.H. Carbon dioxide modified atmospheres (MA) were labeled with 25 (T1), 30 (T2), 35 (T3), 40 (T4), 45 (T5) and 50% (T6) CO_2 by volume, respectively (Shekar *et al.* 2018).

Data collection and analysis

The experiment was maintained with four replications along with controlling under complete randomized design (CRD). Adult mortality data were collected at 24 h intervals up to complete mortality of treated insects. At the end of each exposure time, the bottles were opened, and insects were sieved out and transferred into (9 × 2.5 cm) test tubes having fresh diet. Test tubes were closed with muslin cloth to prevent insect escape and placed in fresh air for up to 24 h. After 24 h, dead insects were sieved out to calculate percent mortality in each treatment. If insects moved, they were considered as live (Annis and Morton 1997).

Corrected percent mortality was calculated using Abbot's formula (Abbot 1925). Results were subjected to analysis of variance (ANOVA) using Statistix-8.1 software and LSD test was performed to compare the means at 5% significance level.

Results

Mortality effect of six modified atmospheres (MA) against *S. oryzae* adult on two diets

The overall mortality percent (%) of *S. oryzae* was significantly ($P \leq 0.05$) influenced by different concentrations of carbon dioxide modified atmospheres concerning time periods. The maximum mortality was recorded in T6 (75.85%) followed by T5 (72.79%), T4 (62.92%), T3 (49.38%), T2 (39.22%) and T1 (27.48%), respectively. Diet also impacted the mortality rate as the maximum mortality% was recorded in insects feeding on wheat (64.89%) while the mortality in insects feeding on maize was (44.32%) recorded. Maximum mortality was assessed after 120 h (88.32%) followed by 96 h (70.91%), 72 h (55.18%), 48 h (38.79%) and 24 h (25.82%) in all treatments. The interactions (Ma × diet) and (Diet × time) were observed non-significant at $P \leq 0.05$. The two factors interaction (Ma × time) and three factors interaction (Ma × diet × time) were recorded significant at $P \leq 0.05$(Table 1).

The mortality (%) increased gradually from T_1 to T_6 and maximum mortality was observed in T_6 from both diets. The mortality% was recorded high on wheat diet as compared to maize (Fig. 1).

In the same way, the interaction between MA × time also resulted in a significant increase in mortality (%). In all treatments, the mortality (%) increased with the passage of time, and maximum mortality was recorded after 120 h (Fig. 2).

Interaction between three factors MA × diet × time positively increased the mortality (%) among the treated adults of *S. oryzae*. The mortality (%) was maximum in T6 and decreased gradually up to T1. In a comparison of diets, maximum mortality was recorded in the insects feeding on wheat diet in all treatments as compared to the insects feeding on maize diet (Fig. 3).

Table 1: Impact of six different modified atmospheres and diets on percent mortality (%) of *S. Oryzae* with respect to time (T1: 25% CO_2), (T2: 30% CO_2), (T3: 35% CO_2), (T4: 40% CO_2), (T5: 45% CO_2) and (T6: 50% CO_2)

Factors	Mortality (%)
Modified atmosphere (MA)	
T_1	27.48E
T_2	39.22D
T_3	49.38C
T_4	62.92B
T_5	72.73A
T_6	75.85 A
Diet (Di)	
Maize	44.32 B
Wheat	64.89 A
Time (T)	
24	25.82 E
48	38.79 D
72	55.18 C
96	70.91 B
120	82.32 A
LSD ($P \leq 0.05$)	
Ma × diet ($P \leq 0.05$)	NS
Ma × time ($P \leq 0.05$)	*
Diet × time ($P \leq 0.05$)	NS
Ma × diet × time ($P \leq 0.05$)	*

Any two means within the column followed same letters are not significant at $P \leq 0.05$. * = significant, NS = non-significant at $P \leq 0.05$

Discussion

Stored products and commodities have been focused to avoid insect pest infestation throughout the world by adopting chemical free strategies (Phillips and Throne 2010). Farmers depend upon the contact insecticide to control the harmful insects of stored commodities. These insecticides are disliked to use due to their hazardous and non-degradable effects on other organism (Morrison 2018). Fumigation is one of the most consistently used methods and several food stuffs are protected by using phosphine and methyl bromide but unluckily some stored product insects have gotten resistance against these chemicals (Wang *et al.* 2000). keeping in mind, the modified atmosphere is one of the best alternative method under a controlled atmosphere (Navarro 2006). CO_2 be the best alternative replacement for phosphine and methyl bromide under stored conditions (Emami *et al.* 2016). The main benefit of this technique is that there is no harmful effect of the CO_2 in the treated products. The level of oxygen can be maintained by vacuum or with the infusion of CO_2 or other gases (Navarro 2006; Conyers and Bell 2007). Temperature significantly affects the mortality (%) *i.e.*, high temperature minimizes the exposure time to get 100% mortality while low temperature enhances the exposure period and also controls the efficacy of CO_2 and other fumigants (Riudavets *et al.* 2009). Utilization of CO_2 fumigation to limit the efficiency of harmful insects are generally productive for stored products (White and Jayas 2003; Pons *et al.* 2010). Our results indicated that the mortality% increased with increase in CO_2 concentration. The previous studies proved that the mortality increased with the enhancement of the modified atmosphere having 20, 40, 60 and 80% CO_2 concentration at 20°C used against *Sitophilus* spp. in maize grains and modified atmosphere comprising 75% CO_2 has fruitful results against *S. oryzae* in different commodities (Carli *et al.* 2010). Insect pests cannot survive in an environment containing more than 35% CO_2 and less than 1% Oxygen level. Our results were in line with the findings of Carli *et al.* (2010) which revealed that mortality rate of exposed insects is enhanced with increased CO_2 concentration on various diets. Annis and Morton (1997) also evaluated the efficacy of different concentrations *viz.*, 15% to 100% CO_2 on the developmental stages of *S. oryzae* in wheat in which

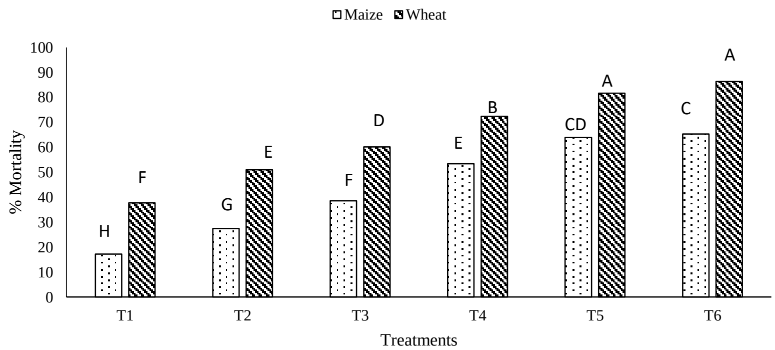

Fig. 1: Impact of interaction between modified atmosphere (MA) and diet on the percent (%) mortality of *S. Oryzae* (T1: 25% CO_2), (T2: 30% CO_2), (T3: 35% CO_2), (T4: 40% CO_2), (T5: 45% CO_2) and (T6: 50% CO_2)

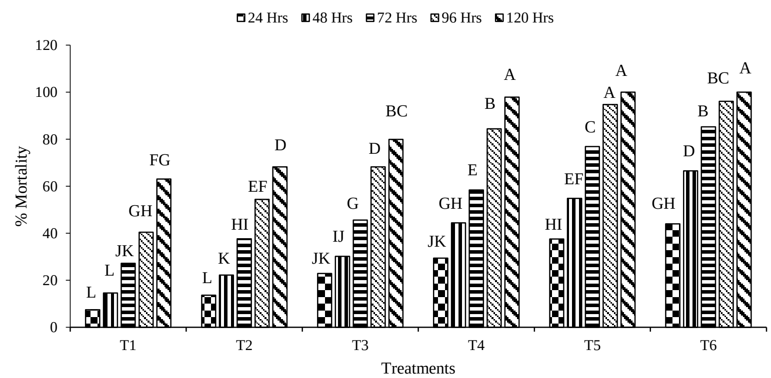

Fig. 2: Impact of interaction between modified atmosphere and time on percent mortality (%) of *S. oryzae* (T1: 25% CO_2), (T2: 30% CO_2), (T3: 35% CO_2), (T4: 40% CO_2), (T5: 45% CO_2) and (T6: 50% CO_2)

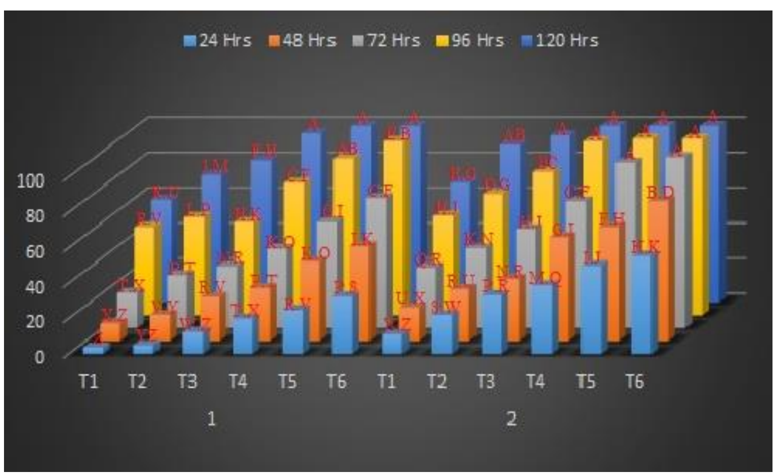

Fig. 3: Impact of interaction between modified atmosphere and diet with respect to time on mortality (%) of *S. oryzae* (T1: 25% CO_2), (T2: 30% CO_2), (T3: 35% CO_2), (T4: 40% CO_2), (T5: 45% CO_2) and (T6: 50% CO_2)

the most tolerant stage was pupae while 100% mortality was obtained within 30 days in egg after exposure. The current work is also co-related with the findings of (Annis and Morton 1997) where death rate at adult stage increased with the enhancement of CO_2 concentration which was followed by death rate at larval stage. Eggs of *Tribolium confusum* and *Tribolium castaneum* are not hatch able at 25% CO_2 atmosphere and high level of nitrogen and low level of oxygen had no effect on feasibility and incubation time, while the enhancement of CO_2 concentration showed significant impact on the incubation period and feasibility (Ali and Lindgren 1970). Our result was also similar with

(Ali and Lindgren 1970) finding which revealed that adult stage was more vulnerable followed by egg, larvae and pupae and in our result also showed that adult stage was more vulnerable than larvae.

Carbon dioxide concentrations have significant effect on mortality of *S. oryzae*.CO_2 showed 56% mortality at 25% CO_2 concentration after an exposure period of 120 h reared on maize diet while mortality was 100% at 50% CO_2 concentration under 50% concentration with in the same exposure time. Similarly, on wheat diet after an exposure period of 120 h, mortality was 58% at 25% CO_2 concentration while mortality was 100% at 45% CO_2 and 50% CO_2 concentrations within the same exposure time. Results showed that toxicity of different modified atmospheres increased by increasing CO_2 gas concentration within same exposure periods. The similar findings were presented by Lindgren and Vincent (1970); Annis and Morton (1997) on two weevil species such as *S. granarius* and *S. oryzae.*

Results indicated that time and concentration posed significant effect on insect mortality reared on two different diets. Insect population reared on wheat diet was more susceptible as compared to maize diet that needs prolonged exposure time to achieve complete mortality. Similarly, increased concentrations decreased the exposure period and vice versa to achieve complete mortality. Present results showed that maximum mortality % was recorded in wheat as compared to maize but in previous study described that *T. castaneum* mortality % increased with the increase of CO_2 concentration and maximum mortality % was recorded in rice (Sarwar *et al.* 2021).

Conclusion

Our results indicated that maximum mortality of *S. oryzae* adult reared on wheat diet required a minimum of four days exposure period at 45% CO_2 concentration. Whereas, on maize diet, the exposure period of five days is required for maximum mortality at 45% CO_2 concentration. It is indicated that maize diet induces vigor in reared insects as compared to wheat diet and increased the exposure time to achieve mortality of exposed insects. The outcomes affirmed that the utilization of high CO_2 fixation in a gastight storehouse and airtight fixed huge sacks is a possible choice to control the event of occurrence during rice storage.

Acknowledgements

Not Applicable

Author Contributions

Zahid Mahmood Sarwar, Planned and supervised the research; Muhammad Mohsan, Zafar Hussain and Muhammad Shahbaz Asghar, conducted the research and wrote the manuscript. Adnan Badar proofread the manuscript and Muhammad Rafique Khan, helped in English editing and final formatting according to journal style.

Conflicts of Interest

All authors declare that they have no conflict of interest

Data Availability

Raw data and Materials are available

Ethics Approval

Not Applicable

Funding Source

Not Available of funds

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