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"EFFECT OF CARBON DIOXIDE TREATMENT AGAINST ANGOUMOIS GRAIN MOTH *SITOTROGA CEREALELLA* (OLIVIER) IN MAIZE"

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ABSTRACT

During storage, maize grains are severely destroyed by insects and other pests. One of the most important causes of grain loss in stored maize is the damage caused by Angoumois grain moth, *Sitotroga cereallela* (Olivier). A study was conducted to evaluate the effect of carbon dioxide treatment against Angoumois grain moth *Sitotroga cerealella* (Olivier) in maize at Seed Research and Technology Centre (S.R.T.C), Rajendranagar, Hyderabad, Telangana during February 2015 to September 2015. The effect of different concentrations of CO_2 *viz*., 20, 40, 60 and 80 per cent against the Angoumois grain moth *Sitotroga cerealella* in maize seed stored for a period of six months was observed. The result revealed that the percentage of seed damage was found to be nil in 60 and 80 per cent concentrations of CO_2 up to six months of storage. Exposure of seeds at 20 and 40 per cent CO_2 concentrations could able to protect the seed from infestation and development of the moth up to four months of treatment but the infestation and progeny development were noticed after five months of treatment. Higher CO_2 concentrations *viz*., 60 and 80 per cent not only protected the seed from infestation and development but also maintain good storability.

KEYWORDS: Carbon Dioxide, Concentrations, Infestation, Maize, Exposure

INTRODUCTION

Storage under MA involves changing the proportions of the normal atmospheric components of the storage enclosure. Research on the effect of different proportions of these atmospheric components against insect pests is an important pre requisite for developing new management techniques which helps in avoiding the use of chemical methods. Application and distribution of CO_2 and N_2 in stored grain bulks or in products stored in containers or fumigation chambers would provide the needed solution to avoiding losses caused by insect pests. Control of serious pests by using chemical insecticides such as malathion, dichlorvos and methyl bromide have disadvantages because of their destructive environmental effects, including depletion of atmospheric ozone, development of resistance in insects and disruption of the food chain (Tuncbilek *et al*., 2009; and Azizoglu *et al*., 2011). Environmental friendly techniques such as aromatic plant extracts and carbon dioxide (CO_2) treatments that do not leave any pesticide residues in stored products have been used against pests as an alternative method (Aliniazee and Lindgren, 1970; Wang *et al*., 2009; Bachrouch *et al*., 2010; and Razavi, 2012). CO_2 has toxic effects on different developmental stages and age-groups of insects. Many researchers have determined the toxic effects of CO_2 according to exposure time to kill the insects (Gunasekaran and Rajendran, 2005). CO_2 has been known as an environmental friendly technique for control of stored-product insects with no residue in food

for a long time (Jayas *et al*., 1991). In view of the above, the present study was planned to assess the effect of carbon dioxide treatment on *Sitotroga cerealella* (Olivier).

METHODS

Mass Culturing of the Test Insect

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Gelechiidae: Lepidoptera) was used as the test insect as it is the major storage pest of maize and other cereals which cause damage both under field and storage conditions accounting for huge losses. Mass culturing of *S. cerealella* was taken up at S.R.T.C, Rajendranagar, Telangana. The mother culture of *S. cerealella* maintained at Seed Entomology Laboratory, S.R.T.C, was obtained and multiplied on locally available maize seeds. For mass culturing, about 50 pairs of adult moths were released with the help of aspirator into plastic containers containing 500 g of disinfested maize seed and the mouth of the container was covered with muslin cloth and tied with rubber bands. Twenty plastic containers were maintained for mass culturing of test insect. The containers were kept undisturbed under ambient laboratory conditions till the emergence of progeny. The newly emerged adults obtained from the culture after 30-35 days of adult moths released were utilized for the maintenance of sub cultures by following the same procedure as described above. Sub culturing of the moth was done at regular intervals to ensure continuous supply of test insects for conducting the laboratory experiments. Thus the pest was mass cultured in the laboratory for 4–5 generations and the freshly emerged adults (1-2 days old) were used in the experimental studies.

Disinfestation of Maize Seed and Determination of Moisture Content of the Disinfested Seed

For conducting the laboratory experiments with CO_2 treatment, types of bags and fabric treatment studies, the freshly harvested maize variety DHM 117 was obtained from Maize Research Station, ARI, Rajendranagar, Telangana. Before subjecting the seeds to experimental study, disinfestation of the seed was done. The seed was disinfested in the hot air oven to rule out the possibility of hidden infestation of the pest from the field by exposing the seed to 50^0C for 4-5 hours. After disinfestation, the seed was kept under laboratory conditions for 2-3 days and the moisture content of the disinfested seed was determined by using “Dicky John” moisture meter. The moisture content of the seed was found to be 10.50 per cent. To increase the moisture content of the seed, the seed was kept in cloth bags and placed in desiccators for about twenty days in which 55 per cent relative humidity was maintained (Solomon, 1951). The required humidity was obtained by dissolving 51 g of potassium hydroxide (KOH) pellets in 100 ml of distilled water and after twenty days the moisture content of the test varieties was determined. The moisture content of the seed was increased to 12.22 per cent.

Sexing of the Insect

The male moth is smaller in size than the female moth. The females were on an average 5.5 mm long and 1.4 mm broad and the male was 5.0 mm long and 1.1 mm broad (Alam,1971). The female had wider wing span than that of the male but fringed wings in both sexes. There were no distinct colour differences of male and female moths. Both the male and female were straw coloured.

The female and male moths were identified based on the descriptions given by Pandey and Pandey, (1976). The sexing was mainly done based on the abdominal characters. The abdomen of the female is creamy, stout with blunt end, where as in male it is blackish, slender with pointed tip when viewed from ventral side.

Effect of Carbon Dioxide Treatment on *S. Cerealella* in Maize

Carbon dioxide treatment was carried out against *S. cerealella* in specially designed airtight containers and the required concentration of CO_2 was released into the airtight containers with the help of carbon dioxide cylinder (Model No. BRG 0/1) fitted with an outlet tube containing a nozzle and a needle. The required concentration of CO_2 to be released into the container was regulated with the help of a pressure nob fitted at the top of the CO_2 cylinder. The required concentration of CO_2 was released into the container with a pressure of 2 kg/cm^2 from CO_2 cylinder.

Design of Airtight Container

The disinfested maize seed of 100 g was placed in airtight plastic containers of 500 g capacity consisting of two perforations of 3 mm diameter which serve as an inlet and outlet holes and nylon tubes of 3 mm diameter were inserted into the holes. Rubber corks of 2.95 mm diameter which exactly seal the inlet and outlet tubes were used to plug them after filling the containers with desired concentrations of CO_2 and thus the entire system was made airtight after releasing the CO_2 into the containers.

Injection of CO_2 into Airtight Containers

Before introducing the CO_2 into the airtight container, the air present in the airtight container was flushed out by opening the outlet present at the top of the container and then it was closed with rubber cork and then the desired concentration of CO_2 was introduced into the airtight container through the inlet located at bottom of the container by injecting the needle of CO_2 cylinder. After injecting the gas, the inlet and outlet tubes were closed at one stroke using rubber corks to prevent escape of CO_2 from the container. To study the effect of CO_2 treatment on *S. cerealella* 120 airtight containers were filled with 100 g of disinfested maize seeds by releasing 5 pairs of *S. cerealella* freshly emerged adults 15 days prior to treatment with CO_2 to ensure uniform level of infestation. After 15 days CO_2 was released at four concentrations *viz*., 20, 40, 60 and 80 per cent with four replications of each treatment and after releasing the desired concentration into the containers they were made airtight by plugging them with rubber corks and sealed with Teflon tape. Twenty containers were maintained for each months. The seed was observed by using sample from 20 containers of all treatments and disposed after 1, 2, 3, 4, 5, and 6 months of treatment. Control was maintained by following the same procedure adopted for the CO_2 studies in plastic containers under normal conditions without exposing the seed to CO_2.

Determination of CO_2 Concentration in Containers after Injection

To ensure whether the desired concentration of CO_2 released in the plastic container was maintained or not, it was checked by using CO_2/O_2 analyzer (PBI Dansensor, PBI 200616, Denmark) to measure the CO_2 concentration in the containers. For determination of CO_2, the analyzer was calibrated with atmospheric air (20.9% O_2 and 0.03 % CO_2) then the needle of the analyzer was introduced into the top outlet tube of the air tight container and the measuring button of the CO_2/ O_2 analyzer was pressed. The concentrations of CO_2 and O_2 present in the air tight container will be displayed on screen within 10 seconds which helps in determining the concentration of CO_2 present in the containers.

The airtight containers containing the disinfested seed exposed to different concentrations of CO_2 were observed after 1, 2, 3, 4, 5 and 6 months of storage by using sample from each container and the following parameters were recorded

- Seed damage (%)

- Adult emergence

Seed Damage (%)

Percentage of seed damage was calculated by taking a random sample of 100 seeds and counting the number of seeds bored holes by *S. cerealella* and converted to percentage.

$$\text{Percentage of seed damage} = \frac{\text{Number of damaged seeds}}{\text{Total number of seeds}} \times 100$$

Adult Emergence

Number of live and dead insects emerged out from 100 g sample seed of each replication of the treatment was counted.

RESULTS AND DISCUSSIONS

Seed Damage

The results obtained from the studies on effect of carbon dioxide treatment on percentage of damaged seeds caused by *Sitotroga cerealella* are presented in Table 1 and graphically depicted in Figure 1.

From the data it was evident those four months after treatment of carbon dioxide on maize seeds which were subjected to artificial infestation of *S. cerealella*, zero infestation was recorded in all the concentrations of CO_2 *viz*., 20, 40, 60 and 80 per cent. The percentage of seed damage in control was found to be 10.75 during first month of storage. After two, three and four months of storage seed damage per cent was 11.88, 15.88 and 18.90 per cent respectively.

However, after five months of CO_2 treatment 4.30 per cent of seed damage was recorded at 40 per cent CO_2 concentration while at 20 per cent CO_2, 6.93 per cent of seed damage was found. In control 20.69 per cent of seed damage was recorded, whereas at 60 and 80 per cent CO_2 concentrations there was zero per cent seed damage and were found to be significantly superior to all the treatments. Similar trend was recorded even after six months of the treatment where in 6.33 per cent of seed damage was recorded at 40 per cent CO_2 which was found to be significantly superior to 20 per cent CO_2 with 7.53 per cent of seed damage. Both the treatments were significantly superior to control where 22.37 per cent of seed damage was recorded. Even after six months of treatment no damage was recorded on seeds at 60 and 80 per cent of CO_2 concentrations.

The results on mean per cent seed damage showed significant differences among the treatments. No damage was observed at 60 and 80 per cent CO_2 concentration which differed significantly from other treatments. The least seed damage of 1.77 and 2.41 per cent was observed at 40 and 20 per cent CO_2 concentrations respectively. The untreated control recorded the highest seed damage of 16.74 per cent.

Results on exposure period revealed that mean seed damage showed increasing infestation with increase in exposure periods. Mean seed damage of 2.15, 2.38, 3.18, 3.78, 6.39 and 7.25 per cent was recorded after one, two, three, four, five and six months of treatment, respectively.

The interaction effect between the treatments and exposure period with respect to seed damage showed that the no damage was recorded in all the treatments up to four months after treatment and also on all the periods of seeds exposed

with 60 and 80 per cent CO_2.

From the results it was evident that CO_2 concentrations of 60 and 80 per cent were fatal to *S. cerealella* while 20 and 40 per cent concentrations of CO_2 though controlled the pest in the initial stages i.e., up to four months but could not protect the seed during prolonged storage of five months and above period. The present investigation are in accordance with the findings of Radhika *et al*. (2014) who opined that exposure of groundnut pods to low concentrations of 10 per cent CO_2 treatment though protected the seed from *Caryedon serratus* up to 3 months but after six months of the infestation increased and went up to 50.79 per cent pod damage. According to Bera *et al.* (2007), all the carbon dioxide concentrations tested (20, 40, 60 and 80 per cent) had completely controlled storage insects (rice weevil (*Sitophilus oryzae*) and lesser grain borer (*Rhyzopertha dominica*)) and storage fungi damage in rice seeds up to 12 months after treatment imposition. Krishnaveni (2012) also reported that exposure of seeds to low concentration of 20 per cent CO_2 treatment though protected the seed up to four months, but after five months of treatment, 12.50 per cent infestation aginst *Callosobruchus chinensis* was recorded and it was further increased to 17.00 per cent after six months.

Table 1: Effect of CO_2 Treatment on Seed Damage by Angoumois Grain Moth, *Sitotroga Cerealella* in Maize during Storage

Co_2 Concentration (%)	Seed Damage (%)						
	1 MAT	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	Mean
T_1-20	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	6.93 (6.94)	7.53 (7.54)	2.41 (5.11)
T_2-40	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	4.30 (5.32)	6.33 (6.34)	1.77 (4.65)
T_3-60	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)
T_4-80	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)	0.00 (4.06)
T_5-Control	10.75 (10.76)	11.88 (11.88)	15.88 (15.89)	18.90 (18.90)	20.69 (20.70)	22.37 (22.38)	16.74 (16.75)
Mean	**2.15 (5.40)**	**2.38 (5.62)**	**3.18 (6.42)**	**3.78 (7.02)**	**6.39 (8.21)**	**7.25 (8.87)**	
	CD(P=0.05)			**Sem ±**			
Concentrations(F_1)	**0.29**			**0.15**			
Exposure period(F_2)	**0.32**			**0.16**			
Interaction($F_1 \times F_2$)	**0.72**			**0.36**			
CV (%)	**7.42**						

MAT – Months after Treatment

Figures in parentheses are angular transformed values

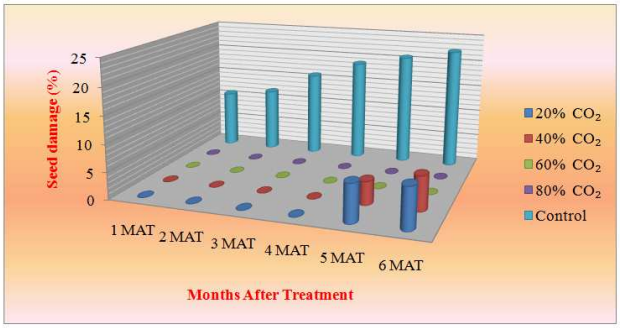

Figure 1: Effect of CO_2 Treatment on Seed Damage (%) by Angoumois Grain Moth, *Sitotroga Cerealella* in Maize during Storage

Adult Emergence

The results pertaining to effect of carbon dioxide treatment on adult emergence of *S. cerealella* are presented in Table 2 and graphically depicted in Figure 2.

From the data it was evident that there was no adult emergence in all the concentrations of CO_2 upto four months of treatment. However, the adult emergence was 13.50 per cent in control after first month of storage which further increased from 24.25 per cent in second month to 41.25 per cent in fourth month after storage.

After fifth month of storage 1.50 per cent adult emergence at 40 per cent CO_2 concentration was observed which was significantly superior to 2.25 adult emergences at 20 per cent CO_2 concentration. Both the treatments were found to be significantly superior to control where 54.25 adult emergences were observed. Similar trend followed after six months after storage with 3.00 per cent adults emerged at 40 per cent concentration of CO_2 which was significantly superior to 5.00 adults emerged at 20 per cent CO_2 concentration. Both the concentrations of CO_2 were significantly superior to control where 62.25 adult emergences were observed.

The results on mean adult emergence showed significant differences among the treatments. No adult emergence was observed at 60 and 80 per cent CO_2 concentrations which differed significantly from other treatments. The least adult emergence of 0.75 was observed at 40 per cent CO_2 concentration and 1.21 per cent adult emergence was recorded at 20 per cent CO_2 concentration. The untreated control recorded the highest adult emergence 37.83 per cent.

Results on exposure period revealed that mean adult emergence goes on increasing with increase in exposure period. Mean adult emergence of 2.70, 4.85, 6.30, 8.25, 11.60 and 14.60 per cent was recorded at one, two, three, four, five and six months after treatment respectively.

The interaction effect between the treatments and exposure period with respect to adult emergence showed that the least adult emergence 0.00 per cent was recorded in all the treatments up to four months after treatment and also on all the periods of seeds exposed with 60 and 80 per cent CO_2.

There was no adult emergence at 60 and 80 per cent concentration of CO_2 up to six months of storage. Hence, 60 and 80 per cent CO_2 concentration was found to be significantly higher than all other treatments. The present investigation are in accordance with the findings of Jayashree *et al*. (2013) there were no adult emergence of *Rhyzopertha dominica* in sorghum seeds up to 315 days when the seed where exposed to 15 and 20 per cent CO_2 concentrations which resulted mortality of insects.

According to Hashem *et al*. (2012b) there was reduction of adult emergence from 4th instar larvae and 3-day-old pupae of *Sitotroga cerealella*, when treated with different CO_2 concentrations and adult emergence also tended to decrease with the increase of CO_2 concentrations in air and exposure period. Further according to Shivaraja *et al*. (2012) pigeon pea seeds treated with CO_2 concentrations of 15 and 20 per cent exposed for 45 days gave cent per cent mortality of *Callasobruchus analis*. They also reported that there was no adult emergence therefore egg laying and mass loss (%) was also nil. According to Jay, (1984) the emergence of immature stages of *Sitophilus oryzae* and *Rhyzopertha dominica* stopped completely from paddy seeds by exposing the adults to 60 per cent CO_2.

Table 2: Effect of CO_2 Treatment on Adult Emergence of Angoumois Grain Moth, S. *Cerealella* in Maize

CO_2 Concentration (%)	Adult Emergence						
	1 MAT	2 MAT	3 MAT	4 MAT	5 MAT	6 MAT	Mean
T_1-20	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	2.25 (1.65)	5.00 (2.34)	1.21 (1.14)
T_2-40	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	1.50 (1.40)	3.00 (1.86)	0.75 (1.02)
T_3-60	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_4-80	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
T_5-Control	13.50 (3.74)	24.25 (4.97)	31.50 (5.66)	41.25 (6.46)	54.25 (7.40)	62.25 (7.92)	37.83 (6.03)
Mean	**2.70 (1.31)**	**4.85 (1.56)**	**6.30 (1.70)**	**8.25 (1.86)**	**11.60 (2.37)**	**14.60 (2.71)**	
	CD(P=0.05)			**Sem ±**			
Concentrations(F_1)	**0.05**			**0.03**			
Exposure period(F_2)	**0.06**			**0.03**			
Interaction($F_1 \times F_2$)	**0.13**			**0.07**			
CV (%)	**4.89**						

MAT – Months after Treatment

Figures in parentheses are square root transformed values

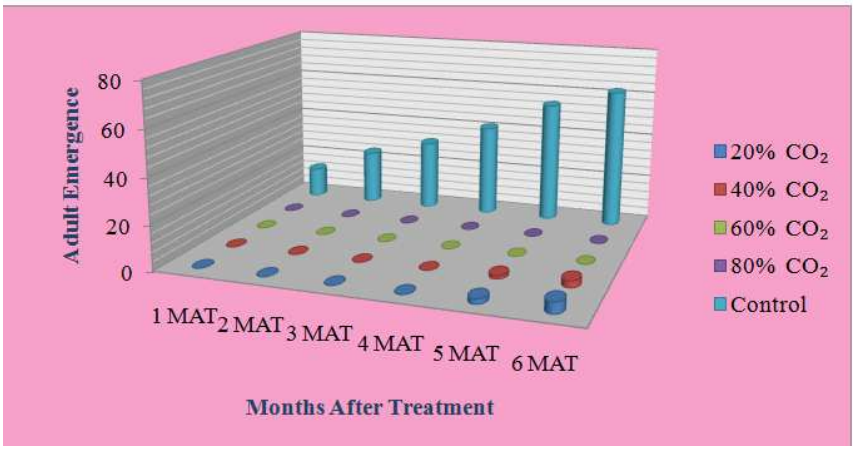

Figure 2: Effect of CO_2 Treatment on Adult Emergence of Angoumois Grain Moth, *Sitotroga Cerealella* in Maize

CONCLUSIONS

The effect of different concentrations of CO_2 *viz*., 20, 40, 60 and 80 per cent against the Angoumois grain moth, *Sitotroga cerealella* in maize seed stored for a period of six months was observed. The results obtained from the study revealed that the percentage of seed damage was found to be nil in 60 and 80 per cent concentrations of CO_2 up to six months of storage. The seed damage decreased with the increase in the CO_2 concentrations and subsequently there was no adult emergence in 60 and 80 per cent CO_2 concentrations, the adult emergence was also nil.

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