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Management of Pulse Beetle, *Callosobruchus maculatus* (F) Population by Nitrogen Based Modified Atmosphere

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ABSTRACT

Callosobruchus maculatus (F) is a direct pest affecting stored green gram causing quantitative and qualitative loss. While other management methods have different side effects on the environment and human health there is a need of ecofriendly methods to manage the pests. Manipulation of the composition of the atmosphere to control stored product insects has a promising future. Studies were conducted to determine the effectiveness of nitrogen based modified atmosphere on *C. maculatus* population. The experiment consisted of two factors *viz*., Oxygen concentration (21, 14, 11, 8, 5 and 2% O_2 with remaining percentage being Nitrogen) and exposure period (3, 5 and 7 days), designed in Factorial Completely Randomized Design (FCRD) with 18 treatments and replicated 3 times. The results showed that insect mortality increased by decreasing the O_2 concentration or by extending the exposure period to low O_2. In addition adult population decreased drastically with decrease in O_2 concentration.

Keywords: stored pests, modified atmosphere, control, Nitrogen

1. Introduction

Manipulation of the composition of the atmosphere to control stored product insects has a promising future (Bailey and Banks, 1975) [1]. This modified atmosphere storage has many advantages compare to conventional methods of storage (Calderon and Barkai-Golan, 1990) [2]. They provide a way to eliminate insects from stored commodities without polluting the atmosphere and are safer traditional fumigants. No harmful residues remain after the treatment of the commodity with nitrogen (N_2) or oxygen (O_2).

Atmospheres which could be utilized for this purpose in grain storage are those with reduced O_2 tension and those with increased CO_2 content or a combination of the two. Means of producing such atmospheres include the purging of airtight silos with N_2 from tankers and the elimination of O_2 using burners or catalytic generators (Navarro, 2006) [3].

Navarro (1986) [4], Banks and Annis (1990) [5] stated that N_2 causes a progressive hypoxia or anoxia when used alone at a high purity level. Generally the lower the O_2 level, the higher the mortality. For effective control, the O_2 level should be less than 3 % and preferably less than 1 % if a rapid kill is required. This effect was shown to be reversed for adult rice weevils, *S. oryzae,* which below 1 % O_2 in N_2 showed tolerance, increasing the lethal exposure time, apparently due to the closure of their spiracles to prevent desiccation (Navarro *et al.,* 1985) [6].

It is known that modified atmosphere is frequently practiced in developed countries (Longstaff, 1994) [7]. There is a need to introduce it in third world countries by the way of studying different gases concentrations to be used. Keeping all these points in view, the present study was initiated to evaluate the effectiveness of Nitrogen based modified atmosphere on *C. maculatus*.

2. Materials and methods

2.1. Study area and the crop used

The experiment was conducted in 2012 in the laboratory of Post-Harvest Technology Centre, (PHTC) Tamil Nadu Agricultural University (TNAU) campus, Coimbatore-3. Green gram seeds (variety CO-6) used for the study were purchased from the Department of Pulses, TNAU-Coimbatore

2.2. Mass culturing of *C. maculatus*

The *C. maculatus* adults collected from stored pulses were utilized for mass culturing. These beetles were reared on fresh green gram seeds that were disinfested by storing in a freezer for one week to kill any insect stages that may be present. About 200 g of green gram seeds were placed in plastic containers, into which approximately unsexed 50 pairs of freshly emerged adults were introduced. The containers were covered with muslin cloth, placed in rearing cage and maintained at a room temperature of 30 ± 5 °C and 70 ± 5% relative humidity (r.h) throughout the period of study. After about 25-28 days, adults that emerged from the culture were utilized for maintenance of sub cultures following the same procedure as described above.

For getting uniform aged adults, 100 g of fresh seeds were exposed to 20 pairs of insects and allowed for one to two days for egg laying. All the adult insects were removed after 1-2 days and uniform aged adults were harvested 25 days after egg laying.

2.3. Experiment Details

2.3.1. Design of Airtight Glass Containers

Glass containers of 500 ml capacity with metal screw cap were used for conducting the experiment on assisted modified atmospheric storage. In the lid, two perforations each of 3 mm diameter were made for inlet and outlet ports. PVC hose of 25 cm length was inserted through a brass hose collar connected to the lid which served as gas inlet and this was connected to the gas mixture apparatus by appropriate tubing, while flushing the gas. Teflon coated silicon septum of 20 mm diameter was inserted into brass check nut and was used to close after filling the gas inside the container which exactly seals the outlet and the entire system was made airtight.

Preliminary studies were done to check the air tightness of the container. Known concentration of gas mixture (CO_2, O_2 and N_2) from the pressurised cylinders was flushed into the glass bottles. The containers were kept for one day and the gas level was checked again with the help of O_2 analyzer to confirm the gases initial level.

2.3.2. Design and Layout of Experiment

The experiment consisted of two factors *viz*., different concentrations of O_2 (21% O_2 as control), 14% O_2, 11% O_2, 8% O_2, 5% O_2 and 2% O_2) and exposure period (3, 5 and 7 days), designed in Factorial Completely Randomized Design (FCRD). The treatment combination gave 18 treatments and each replicated 3 times and in total 54 experimental units.

2.3.3. Release of Nitrogen Gas into an Airtight Glass Container

Nitrogen gas was released into an airtight glass container at different concentration to get low O_2 atmosphere. Containers were maintained for different period of storage (3, 5 and 7 days). Fifteen numbers of 1-2 day old, unsexed adult pulse beetles were transferred to the 500 ml experimental glass bottle containing 200 g of green gram and N_2 gas from cylinder was introduced. The airtight containers were provided with 3 mm inlet hole designed in the lid to flush the gas.

While purging N_2 gas, the outlet port was kept open to flush out the air from the container and the time required to get desirable concentrations of O_2 in N_2 was determined (for 14%, 11%, 8%, 5% and 2% O_2 concentrations, the time required was 2, 4, 5 and 8 minutes respectively). After injecting the N_2 gas, the inlet and outlet ports were closed at one stroke using a small pinch clip and brass check nut respectively to prevent escape of the gas from the container. The concentration of O_2 gas in the container was determined using O_2 analyzer.

2.3.4. Observations and Data Recorded

i. Adult Mortality

Per cent mortality was calculated using the following formula.

Per cent adult mortality = No. of adults dead/ No. of adults released X 100. In case of insects mortality in the control, Abbot's correction was applied (Abbot, 1925)[8] using the following formula.

$$\text{Corrected mortality} = \frac{\text{Observed \% mortality in treatment} - \text{Observed \% mortality in untreated check}}{\text{100- Pc observed per cent mortality in untreated check}} \times 100$$

ii. Progeny development

After recording the adult mortality, in the same sample, the number of eggs laid was counted from 100 randomly selected grains per replication for each treatment. Observations were made for the exposure periods of 3, 5 and 7 days. New adults were recorded starting from twenty five days after egg laying till there was no new adult emergence for each treatment. The per cent egg mortality was also calculated based on the number of adults emerged (Howe, 1971) [9].

Per cent egg mortality = 100 – Per cent egg survival. With
Per cent egg survival = No. of adults emerged / No. of eggs laid X. 100.

2.3.5 Statistical Analysis

The data obtained from different experiments were statistically analyzed by analysis of variance (ANOVA) by SAS 9.1.3 Portable software. Data on adults *C. maculatus* and eggs mortality were transformed using arc sine ($\sin^{-1}\sqrt{p}$) while egg laying and adult emergence were transformed using square root ($\sqrt{x}+0.5$) The mean separation was done by Least Significance Difference (LSD) test. In case of mortality in the control, the percentage mortalities were subjected to correction as developed by Abbot (1925) [8].

3. Results and Discussion

3.1. Adult Mortality

The results of the table 1 showed that there was significant difference in the mortality of pulse beetle by counts made at 3, 5 and 7 days of exposure. The adult mortality was found to be high (91.9%) at 2% O_2 when compared to other concentrations. At 14%, 11%, 8% and 5% O_2 concentration, the mean per cent adult mortality recorded was 51.0, 63.5, 73.3 and 83.5 respectively. In addition, the exposure period influenced significantly the adult mortality of *C. maculatus*.

Among the three exposure periods, the highest mortality was recorded at 7th day with 71.8%. The insect mortality increased by decreasing the O_2 concentration or by extending the exposure period to low O_2.

Storey (1975) [10] stated that during the N_2 atmosphere exposure period, insects consume less O_2 to avoid the effect of anoxia. In case of anoxia, the development of insects virtually ceases and survival depends only on the capacity to accumulate glycolytic products and to reduce metabolism needs. It was found that for the same O_2 concentration, if duration of exposure increased the total mortality per cent also increased. Babu *et al.* (1989) [11] recorded an increase in mortality of *C. chinensis* with the increase in exposure period.

Table 1: Effect of nitrogen based modified atmosphere on adult mortality of *C. maculatus*

O_2 concentration	Per cent adult mortality after different exposure periods			
	3 days	5 days	7 days	Mean
N1 - 14% O_2	41.5	44.8	66.7	51.0[e]
N2 - 11% O_2	51.3	62.4	76.8	63.5[d]
N3 - 8% O_2	63.2	75.1	81.7	73.3[c]
N4 - 5% O_2	73.0	85.1	92.2	83.5[b]
N5 - 2% O_2	82.9	92.8	100.0	91.9[a]
N6 - Control	4.4	8.9	13.3	8.9 [f]
Mean	52.7[c]	61.5[b]	71.8[a]	
	Gas concentration	**Periods**	**Interaction**	
CD (0.05)	4.3	3.0	NS	
SE (d)	2.1	1.5	7	
CV%	8.0			

NS - Not significant
Means followed by the same letters are not significantly different at *P*=0.05 (LSD)

3.2. Progeny development

i. Eggs laying

The treatments were significantly different from one another and 2% O_2 concentration was most effective with less number of eggs (Table 2).

Table 2: Effect of N_2 based modified atmosphere on egg laying by *C. maculatus*

O_2 concentration	Mean No. of eggs laid after different exposure periods			
	3 days	5 days	7 days	Mean
N1 - 14% O_2	8.0	14.0	14.0	12.1[b]
N2 - 11% O_2	5.7	8.0	9.7	7.8[c]
N3 - 8% O_2	4.7	5.7	7.3	5.9[d]
N4 - 5% O_2	2.7	3.0	3.7	3.1[e]
N5 - 2% O_2	1.7	2.3	2.3	2.1[f]
N6 - Control	17.7	20.3	21.3	19.b[a]
Mean	6.7 [b]	8.9[a]	9.8[a]	
	Gas concentration	**Periods**	**Interaction**	
CD (0.05)	0.2	0.1	NS	
SE (d)	0.1	0.0	0.2	
CV%	7.9			

NS - Not significant
Means followed by the same letters are not significantly different at *P*=0.05 (LSD)

For the exposure period, there was significant difference on egg laying by *C .maculatus* and the mean number was high at 7th day (9.78). The number of eggs increased with the increase in O_2 concentration and exposure time. Spratt (1984)[12] showed that in case of *T. castaneum*, egg production declined with decreasing O_2 concentration ranging between 20- 5%.

ii. Adult emergence

The results revealed that the O_2 concentration influenced significantly the adult emergence. Adult population decreased with decrease in O_2 concentration (Table 3).

Table 3: Effect of N_2 based modified atmosphere on adult emergence of *C. maculatus*

O_2 concentration	No. of adults emerged after different exposure periods			
	3 days	5 days	7 days	Mean
N1 - 14% O_2	6.3	10.3	8.0	8.2b
N2 - 11% O_2	4.3	5.3	4.6	4.8c
N3 - 8% O_2	2.7	2.6	3.3	2.9d
N4 - 5% O_2	1.0	1.0	1.0	1.0e
N5 - 2% O_2	0.7	0.6	0.0	0.4f
N6 - Control	17.3	19.6	20.3	19.1a
Mean	5.4	6.6	6.2	
	Gas concentration	**Periods**	**Interaction**	
CD (0.05)	0.2	NS	NS	
SE (d)	0.1	1.0	0.2	
CV%	9.9			

NS - Not significant
Means followed by the same letters are not significantly different at P=0.05 (LSD).

In the control, the adult emergence from grains reached a mean value of 20.33 adults after 7 days while in grains exposed to 2 % O_2 concentration there was no adult emergence. In the control, the adult emergence from grains has reached a mean value of 20.3 after 7 days while in 2% O_2 concentration there was no adult emergence. There was no significant difference with reference to exposure period on the adult emergence.

iii. Eggs mortality

The effect of O_2 level on egg mortality is given in the Table 4.

Table 4: Effect of N2 based modified atmosphere on egg mortality of C. *maculatus*

O_2 concentration	Per cent egg mortality after different exposure periods			
	3 days	5 days	7 days	Mean
N1 - 14% O_2	20.5	25.7	44.6	30.3[d]
N2 - 11% O_2	22.8	33.1	51.8	35.9[d]
N3 - 8% O_2	43.3	54.3	56.1	51.2[c]
N4 - 5% O_2	61.1	66.6	72.2	66.6[b]
N5 - 2% O_2	66.6	72.2	100	79.6[a]
N6 - Control	2.4	3.3	4.7	3.4[e]
Mean	36.1[b]	42.5[b]	54.9[a]	
	Gas concentration	**Periods**	**Interaction**	
CD (0.05)	8.89	6.29	NS	
SE (d)	4.38	3.10	7.59	
CV%	22.57			

NS - Not significant

Means followed by the same letters are not significantly different at *P*=0.05 (LSD)

The results indicated that there was significant (P<0.05) difference in egg mortality considering the O_2 concentration and exposure period. For the influence of O_2 concentration, it was found that at 2% O_2 concentration the mean per cent egg mortality was 79.6 while in the control egg mortality was low with 3.46 %. For the exposure period, the mean per cent egg mortality was high at 7th day and low at 3rd day with 54.91 and 36.15 respectively.

Adult population decreased drastically with decrease in O_2 concentration. This may be due to the low number of survived eggs and the effect of N_2 on egg hatching. Spratt (1979) [12] also reported that the numbers of F_1 adults were reduced when either oviposition or development or both took place in modified atmosphere with O_2, CO_2 or in the gas mixture.

Laboratory tests by Conyers (2006) [13] showed that under O_2 level of 3-5 %, no population increase was observed for *S. granarius*, *S. oryzae* and *T. castaneum*. In addition the the adults were more susceptible to reduced O_2 level than the eggs by comparing the % mortalities achieved. This finding is in agreement with Campbell and Sinha, (1978)[14] who found that the egg stage of stored product insects is the most resistant stage to fumigants since they respire at low rate than larvae or adults

4. Conclusion

Nitrogen based atmosphere storage with low oxygen concentration (2%) showed an impact on progeny development. The number of eggs laid was low compared to control (normal atmosphere) and there was no adults emerged after 7 days of exposure. Nitrogen based modified atmosphere could be one of the promising and un-risky methods for pulse beetle management.

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