

Hermetic Storage Negatively Affects the Respiratory Metabolism of Groundnut Bruchid *Caryedon serratus* (Olivier) and thereby it's Control

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ABSTRACT

Groundnut is an important food legume and an oilseed crop which is prone to attack by several insect pests during pre and post-harvest stages. Insect pest management in groundnut is usually done by chemicals. The practice though effective, it is not an ideal option as the commodity's direct consumption is increasing among public owing to its nutritious profile. Groundnut bruchid, *Caryedon serratus*, is an important storage pest of groundnut. An alternate and chemical free method for the management of storage pests in groundnut is practice of hermetic storage. The effective use of the technology can be done by determining the mechanism and process underlying it by studying the respiratory metabolism of an insect. It was found to consume about 39.97 ml of oxygen during its development from egg to pupa and released 26.21 ml of carbon dioxide. The respiratory quotient values were found to be 0.53, 0.68 and 0.64 at its egg, larval and pupal stages respectively. The information of respiratory quotient of an insect and its oxygen requirement for growth and carbon dioxide emission during development help to design effective hermetic storage management techniques.

Key words: Groundnut, *Caryedon serratus*, Respiratory Quotient, Hermetic storage

INTRODUCTION

The major challenge for many of the developing nations throughout world is ensuring food availability for the exponentially increasing population. It is estimated that world population will reach 9 billion by 2043 which means that food production must increase by at least 50 per cent in the next 40 years to be able to feed them all. Researchers are focusing on traditional breeding, genetic engineering, and increasing the agricultural

land use etc. for achieving food security. It is suggested by many that increase in food production alone will not ensure food security but a substantial reduction in post-harvest losses will help in attaining the objective. It has been aptly said that 'a grain saved is a grain produced' and this is the central motive behind all the technologies that have been developed or are being developed to reduce post-harvest losses.

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Many technologies are being developed to eradicate post-harvest losses. Among many technologies developed, the concept of management of storage pests based on hermetic technology occupies a significant place as it uses no harmful chemicals and is also economically cheap that facilitates adoption by the small and marginal farmers especially in Asia and sub-Saharan Africa.

In groundnut, bruchid *Caryedon serratus* (Olivier) is an economically important storage pest and causes severe damage when improper storage conditions arise. Though most of the storage insect pests attack kernels, *C. serratus* is the only major pest of groundnut that infests unshelled pods as well as kernels. Post-harvest losses in groundnut ranges between 10 to 25% of the production in Asia and a pod damage by bruchids up to 83% has been reported under unprotected conditions of groundnuts following 8-13 months of storage². Insect infestation not only causes direct loss to the produce, but also creates entry points to the fungal colonization especially *Aspergillus flavus* group of fungi which upon invasion, results in decreased level of germination, decreased kernel weight, kernel discoloration, chemical and nutritional changes in addition to aflatoxin contamination⁸. Based on the severity and extent of damage caused directly and indirectly by the groundnut bruchid many management strategies have been designed from time to time but fundamentally it is to be taken into consideration that in general for any grain ecosystem, the most important abiotic conditions influencing insect attack, mold growth and mycotoxin production are water activity (a_w), temperature, and gas composition⁴. Hence the present study is planned with an objective to understand the respiratory biology of *C. serratus* which forms a basis to know the dynamics of hermetic management as recent studies report Purdue Improved Crop Storage (PICS)-based triple layer plastic bags offer protection against groundnut bruchid⁷.

Hermetic storage technology is nothing but usage of sealed containers to

protect stored grain against insect pests and mold fungi. It works on the principle of oxygen depletion by insects already present in the grain when it is put in to storage. When the O₂ level in the container falls low enough the insects cease feeding, growing and developing leading to the arrest of population expansion. The container also offers a protective barrier against further invasion by pests coming from the outside as well as cross infestation to the other stores nearby. As the technology is based mainly on the consumption of available oxygen by the insects for its survival growth and development which can be other way related to the respiratory metabolism of an insect. However, the technology is more often being evaluated by researchers as an effective storage pest management tool and efforts on understanding certain other factors that if identified could enhance the performance of technology is given least importance. Hence it is to be noted that not only assessing the performance of the technology against different insects on different stored produce but understanding the dynamics behind the process of hermetic storage is much important. Some of the basic things which one should know for understanding the mechanisms to make the technology to be effectively utilized under different conditions, for different pests in different commodities includes, how much oxygen does an insect consume; what is the threshold for oxygen levels at which the insects stops feeding and become inactive. Further, whether all the stages of the insect equally affected by lowered oxygen concentrations (hypoxia) or do different stages vary in their susceptibility to hypoxia; after what period of time and at what rate do the insects die after the container is sealed; what are the effects of temperature and humidity on survival, among others. In the present study an effort has been put to gain knowledge on the fundamental aspect of how much oxygen a groundnut bruchid consume at different stages of its life cycle and its respiratory metabolism has been estimated which acts as a foundation for use of hermetic technology against the insect *C. serratus*.

MATERIALS AND METHODS

Initial culture of groundnut bruchid, *C. serratus* (Olivier), was collected from naturally infested pods stored in the godowns of groundnut breeding unit at ICRISAT, Patancheru. The bruchid population was then multiplied under laboratory conditions at a temperature of $25\pm 2^{\circ}\text{C}$ and 70 % relative humidity using groundnut pods of variety ICGV02266. The bruchids population was maintained in the plastic jars (15 cm X 10cm diameter) fitted with fine mesh lids to provide good ventilation and aeration. A pair of freshly emerged day old male and female were separated by sexing and were placed in a plastic jar containing very few groundnut pods. The insects were observed carefully for oviposition and soon after oviposition the pods containing only single egg on it were identified which were separated and put them in an air tight septum bottles (Sigma Aldrich). Ten such pods each containing single zero day old egg was placed one each in air tight septum bottles of 240 ml volume and ten similar bottles with a normal pod without egg was placed which serves as a control to determine the oxygen consumption by the pod. The septum bottles were kept in dark place at temperature of $25\pm 2^{\circ}\text{C}$ and 70 % relative humidity.

The initial oxygen and carbon dioxide concentrations in the septum bottles were recorded and there after the changes in O_2 and CO_2 concentrations were measured at every 12 hours interval using a Mocon PAC Check® Model 325 head space analyzer (Mocon, Minneapolis, MN, USA). The mean data obtained from the ten control septum bottles was subtracted from the mean data obtained from the ten septum bottles containing eggs so as to avoid the respiration performed if any by the pod. The septum bottles were regularly observed to determine the transformation of egg to larva, larva to pupa and pupa to adult so as to quantify the amount of oxygen consumed by the insect for transforming from one particular stage to the next. The quantification

was done by subtracting the initial oxygen concentration from the concentration of oxygen measured just after the transformation of an insect stage. Similarly the CO_2 concentration generated was quantified by subtracting the concentration of the CO_2 measured just after the transformation of the insect from the initial CO_2 concentration. The data obtained from all the ten septum bottles was recorded and mean value generated from it was used for calculation. Similar pattern was followed to determine the quantity of oxygen consumed and Carbon dioxide released by the insect while transforming from one stage to the other. The entire data was summed up to determine the total quantity of oxygen used by the insect for completion of life cycle and was represented in the form of volume. Based on the volume of septum bottle (240 ml) the percentages of O_2 and CO_2 were converted into ml using a conversion factor which vary depending up on the volume of the septum bottle collected. Once the O_2 and CO_2 were determined they were used to calculate the Respiratory quotient value following the formula³.

$$\text{RQ} = \text{CO}_2 \text{ produced (ml)} / \text{O}_2 \text{ consumed (ml)}$$

RESULTS AND DISCUSSION

The results suggest that a single bruchid consumed about 5.44 ml of oxygen for completion of egg to first instar stage, releasing 2.9 ml of carbon dioxide. Further it consumed the highest quantity of oxygen (32.97 ml) for its development from first instar to final instar releasing 22.68 ml of carbon dioxide. Relatively low quantity of oxygen (1.56 ml) was used by bruchid for its development from final instar to pupal formation with release of 0.64 ml of carbon dioxide (Table 1). A total of 39.97 ml of oxygen was consumed by the bruchid for its development from egg to pupa and simultaneously released 26.21 ml of carbon dioxide during the process.

The respiratory quotient (RQ) calculated from the data obtained on the quantity of oxygen consumed and carbon dioxide released at each stage showed the highest RQ value of 0.68 for the development stage starting from first instar to final instar. On the other hand, low RQ value was recorded for formation of pupa from final instar larva. RQ value of 0.53 was recorded for the development of bruchid from its eggs to first instar (Fig.1). The observation of highest RQ value during larval development compared to egg and pupal development was due to production of lipids from carbohydrates⁶. The insects usually get the energy by burning the carbohydrates and transformed them into lipids during active feeding larval stages wherein they consumed high oxygen resulting in higher RQ values¹. The results obtained in the present study helps to understand the respiratory metabolism of groundnut bruchid survival. These dynamic changes in the O₂ and CO₂ levels that occur within a sealed storage container cause conditions of hypoxia (reduction in availability of oxygen) and hypercarbia (increase in carbon dioxide concentrations) which result in cessation of feeding there by growth and development, ultimately leading to death of insect. The level at which the conditions of hypoxia or hypercarbia occur can be determined with the knowledge of actual oxygen requirement by the insect and its availability in the container.

These parameters could be different for different insects which can be clearly explained taking example of two storage insects infesting groundnut. The groundnut if placed in the form of pods in a given container having more of head space that certainly contain more of oxygen above and also in the spaces between the pods. If such a container is evaluated for its ability to contain groundnut bruchid multiplication that will give a scope to better understand the respiratory metabolism of *C. serratus* in spite of presence of large quantity of oxygen. The reason could be that

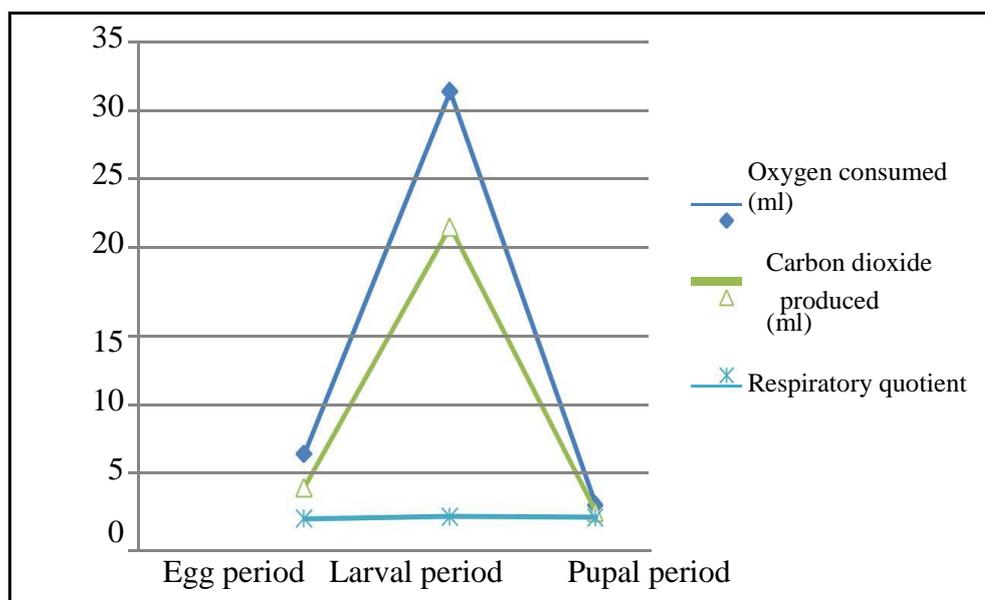
bruchid being a bigger insect and having long life cycle that correlates with high respiratory metabolism as much of the available oxygen will be utilized by small number of insects present in the container without giving much scope for next generation of insects. However the same container if infested with *Tribolium* may not give encouraging results as the insect is smaller, its life cycle is short and its respiratory metabolism may be less giving much scope for the insect to reproduce more as availability of oxygen will be never a limiting factor for it in a given period of time.

However, few studies have revealed that many of the storage insects continue feeding normally at extremely high levels of carbon dioxide, the levels which are far above those that would kill a mammal within seconds. Thus, it is to be understood clearly, that feeding activity falls in response to the drop in O₂ and not to the rise in CO₂ concentrations. These findings support use of air tight containers or materials which do not permit exchange of gases as effective sources of hermetic technology rather than a modified atmosphere storage technology where in high concentrations of carbon dioxide is pumped in to achieve insect mortality.

The findings which propose hypoxia conditions leads to insect mortality gains support from the fact that oxygen insufficiency causes two major problems in insects. First, the insect finds itself unable to utilize oxidative metabolism to form the ATP essential for normal body functions of maintenance, growth, development and movement. Second, the low levels of O₂ propel development of reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide, within insect body that can damage membranes and interfere with proteins and enzymes of various metabolic activities. The only way to circumvent this happening by the insect is to shut down all major metabolic activity, thereby reducing the levels of the ROS species⁴.

Table 1: Oxygen consumption at different developmental stages of groundnut bruchid, *C. serratus*

| Stage of life cycle | Duration (Days) | Oxygen consumed (ml) | Carbon dioxide released (ml) | Respiratory Quotient |
|---------------------|-----------------|----------------------|------------------------------|----------------------|
| Egg period | 5-7 | 5.44 | 2.90 | 0.53 |
| Larval period | 15-18 | 32.97 | 22.68 | 0.68 |
| Pupal period | 12-14 | 1.56 | 1.00 | 0.64 |

**Fig. 1: The quantity of oxygen consumed and carbon dioxide released at different developmental stages of groundnut bruchid, *C. serratus***

CONCLUSION

Thus it is suggested from the above facts that conditions leading to hypoxia result in much faster insect mortality than hypercarbia and designing of conditions which create hypoxia and hypercarbia simultaneously will be a better option for adopting it in storage pest management following hermetic technology. This could be achieved by knowledge on selection of packaging materials or containers, the type and nature of storage insect that infests the produce its respiratory metabolism and proper filling of the container giving less scope for presence of gases and storing of sufficiently cleaned produce. The other important point that has to be noted that whether the produce is stored for shorter duration or longer duration and whether it is stored for seed purpose or for consumption. If

stored for seed purpose the low oxygen and high carbon dioxide for a longer period of time could affect seed germination.

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