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Oxygen requirement of dolichos bean bruchids at different stages of its life cycle

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Abstract

The present investigation was carried out from September, 2017 to March, 2018 in the research laboratory of College of Horticulture, Rajendranagar, Hyderabad, SKLTSHU. Oxygen requirement of *Callosobruchus theobromae* at different stages of its life cycle and performance of triple layer bags as a source of hermetic storage technology for management of pulse bruchid in Dolichos bean seed was investigated. The respiratory quotient (RQ) calculated revealed that a single bruchid for completing life cycle from egg to pupal stage used 39.97 ml of oxygen and 26.21 ml of carbon dioxide simultaneously. The conditions in triple layer PICS bag were less than RQ at all stages with less than critical oxygen levels required for bruchid survival.

Keywords: Oxygen requirement, dolichos bean bruchids, stages of bruchid life cycle

Introduction

Dolichos bean (*Lablab purpurea* L.) also called as Dolichos bean or hyacinth bean belongs (Fabaceae) is a native of India and mainly cultivated as a inter crop with cereals. It is also called as Bonavist bean, Egyptian kidney bean, Egyptian bean, Hyacinth bean, Field bean, Indian bean, Indian butter bean. In South India this crop is best grown for fresh green pods used as vegetable and dry seeds for preparations of various dishes and the other plant parts as fodder for livestock. The green pods contain vitamin A (864 IU), vitamin C (12.6 mg), protein (31.58%), iron (8.89 mg) and rich in calcium (78 mg). The dried seeds contain protein (40%), carbohydrates (20.04%) and fat (1%).

Dolichos bean major producing states are Uttar Pradesh, Telangana, Madhya Pradesh, West Bengal, Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra, Haryana and Kerala. Legumes are rather unique compared to other vegetables in that they can obtain free atmospheric nitrogen through their symbiotic association with the nitrogen fixing bacteria. The nitrogen fixed in the root nodules are not only available to the plant but they also enrich the soil, in varying amounts, when the plants complete their life cycle. Indian bean is one of the most popular perennial vegetable crop in India. The nutritive quality of dolichos bean is better than that of French bean (Aykroyd, 1963) [1]. The ripe seed contains high protein (Schaaffhausen, 1963) [13]. The crop suffers losses in the field as well as in the storage. The bruchid or infested pulses are rendered unfit for consumption because of the presence of excreta and metabolic waste products like uric acid which leads to fungal infection of the grains (Gowda and Kaul, 1982) [6].

Bruchids also called as pulse beetle are distributed throughout the world except Antarctica and the largest number of species occurs in tropical regions of Asia, Africa and South America (South, 1979) [15]. There are approximately 1300 described species of bruchids in the world and as many again to be described (Johnson and Kistler, 1987) [8]. About 84% of the known hosts of bruchids are in the family Leguminosae (Johnson, 1970) and 33 families have been reported as hosts (Kinslover, 1979) [10]. PICS are the latest hermetic storage technology gathered magnumous response over world. Emekci *et al.* (2004) [5] reported that mortality of insects is caused at low levels of O₂ (5%) which caused metabolic stress in insects due to increased respiration rate. Conyers and Bell (2007) [4] revealed that the modified atmosphere containing oxygen less than 5% and carbon dioxide more than 10-20% at a temperature of 20 – 25 °C and relative humidity of 75 - 85% caused poor emergence of adults and sometimes led to adult mortality with minimum oxygen requirement of five coleopteran storage pests *C. ferrugineus*, *O. surinamensis*, *S. granarius*, *S. oryzae* and *T. castaneum*. Cheng *et al.* (2012) [3] observed the impact of hypoxia and hypercarbia conditions on cowpea bruchids, when exposed to two different combinations of O₂/CO₂ concentrations *viz.*, 10% O₂+10% CO₂ and 2% O₂+18% CO₂ and recorded egg to adult mortality at 2% O₂+18% CO₂ concentration.

Triple layer PICS bags work as do other hermetic storage containers such as sealed steel drums (Seck *et al.* 1996) [14] because insects respire aerobically and thus utilize the oxygen in the airtight container while also raising CO₂ levels. Once the oxygen level in the container falls sufficiently low, insects cease feeding and become inactive (Margam, 2009) [11]. Inactivity itself causes the growth and development to cease and in turn reproduction stops. This results in the arrest of pest population growth. During the oxygen deficit caused inactivity insects begin dying. The early instar larvae and pupae appear to be particularly vulnerable; keeping the above in view, the present investigation was carried out to estimate the oxygen requirement of dolichos bean bruchids at different stages of its life cycle.

Material and Methods

The investigation was carried out from September, 2017 to March, 2018 in the research laboratory of College of Horticulture, Rajendranagar, Hyderabad, and SKLTSU.

Estimation of Oxygen requirement of *Callosobruchus theobromae*

The total oxygen requirement of the pest *Callosobruchus theobromae* for completion of its life cycle i.e., from the egg laid to its emergence as adult was estimated using air tight septum bottles (Sigma Aldrich). Ten dolichos bean seeds containing single zero day old egg was placed one each in air tight septum bottles of 240 ml volume and ten similar bottles with normal healthy dolichos bean seeds without any eggs containing on it was taken.

The septum bottles with eggs on seed and normal seed without eggs was kept in at a temperature of 25±2°C and 70% RH. The initial oxygen and carbon dioxide and changes in their concentrations in septum bottles were recorded and for every 12 hours interval using a Mocon PAC Check Model 325 head space analyser (Mocon, Minneapolis, MN, USA). The mean data obtained from the ten check 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 consumes by the insect for transforming from one particular stage to the next. The quantification was done by subtracting the initial oxygen concentration of oxygen measured just after the transformation of an insect stage. Similarly the CO₂ concentration generated was quantified by subtracting the concentration of CO₂ measured just after the transformation of the insect from the initial CO₂ 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 its life cycle and was represented in the form of volume. The O₂ and CO₂ concentration were converted in to volume by using the following formula.

Respiratory Quotient (RQ) value was calculated by using formula (Jakobsen and Thorbek, 1993) [7]

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

Oxygen (O₂) and Carbon dioxide (CO₂) concentrations:

Oxygen (O₂) and Carbon dioxide (CO₂) concentrations were recorded using a Mocon PAC Check 183 Model 325 head space analyzer (Mocon, Minneapolis, MN, USA).

Results and Discussion

The oxygen requirement for complete life cycle of bruchid was studied. The results showed that single bruchid consumed about 5.44 ml oxygen for completion of egg to first instar stage, releasing 2.9 ml of carbon dioxide and from first instar to final instar released 22.68 ml of carbon dioxide. Final instar to pupal formation released 0.63 ml carbon dioxide. The bruchid released 26.20 ml of carbon dioxide and 39.96 ml of oxygen was consumed. The study explained the mode of action of hermetic storage and its effect on dolichos bean seed bruchid survival. The changes in hermetic storage *i.e.* oxygen (O₂) and carbon dioxide (CO₂) levels in closed bag causes the increase in CO₂ level and decrease in O₂ level which cause death of the pest at that hypercarbia and hypoxia condition. Insects obtain the energy by burning the carbohydrates and transformed it into lipids during active feeding larval stages wherein they consume high oxygen (Bhattacharya *et al.* (2003) [2]. The feeding activity of pest reduces due to drop in oxygen concentration but not due to rise in carbon dioxide concentration. Due to low oxygen levels the insect has the only way is to stop all its metabolic activities (Margam, 2009) [11].

Hypoxia conditions lead to insect mortality and causes two major problems in insects. First the insect itself finds unable to utilize oxidative metabolism to form the ATP essential for normal body functions of maintenance, growth, development and movement, etc. Second, the low levels of oxygen 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 triple layer PICS bag which creates hypoxia (reduced oxygen levels) and hypercarbia (increased carbon dioxide levels) simultaneously will be a better option for management of storage pest.

The respiratory biology of *Callosobruchus theobromae* revealed that pulse beetle required about 39.96 ml of oxygen and 26.20 ml of carbon dioxide simultaneously for its development from egg to pupal stage. The respiratory quotient (RQ) at different life stages showed highest RQ for development from first instar to final instar. Creating hypercarbia and hypoxia condition will lead to death of bruchid. The respiratory quotient conditions in the triple layer PICS bag storage were less than RQ at all stages with less than essential oxygen levels required for bruchid survival. The results are in line with the reports of Murdock *et al.* (2012) [12], who estimated that 8.9 ml of oxygen required for completion of life cycle by an individual cowpea bruchid, *C. maculatus* and observed that there is cessation of feeding demonstrated activity due to drop in oxygen concentration by about 2-3 percent by the bruchid. The bruchid required certain amount of oxygen for its growth, development and completion of its life cycle.

From the results, the RQ of 0.53 was registered. Hence, maintaining RQ in the storage exist below 0.53 will keep the produce free from infestation of pulse beetle. Creation of hypercarbia and hypoxia conditions leads to mortality of the insect.

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