THE SIGNIFICANCE OF FREE AIR CO\textsubscript{2} ENRICHMENT AND OPEN ROOF VENTILATION GREENHOUSE SYSTEMS IN A STUDY OF MEALWORM BEETLE, 
*Tenebrio molitor* L. (COLEOPTERA: TENEBRIONIDAE)

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**ABSTRACT**

*Tenebrio molitor* L. (Coleoptera: Tenebrionidae) is an insect storage pest that has been used as a subject in Integrated Pest Management (IPM) research. The aim of this study is to determine the importance of conducting insect-related studies, especially on *T. molitor* under a Free Air CO\textsubscript{2} Enrichment (FACE) System and Open Roof Ventilation Greenhouse System (ORVS). FACE system provides a natural microclimate and biotic interactions, while ORVS is an artificial environment with regulation of its environmental parameters. More accurate comparisons can be made to the results obtained under the similar environmental factors including elevated CO\textsubscript{2} concentration. Based on the results, the mortality time of *T. molitor* adults in ORVS (5-6 weeks) is the fastest, followed by FACE (9-10 weeks) and RR as a control (11-12 weeks). The highest significant time difference shows by the last adult individual dead is between ORVS versus RR is 6 weeks. Therefore, mortality rate of *T. molitor* adult and their life span are directly proportional to the elevated CO\textsubscript{2} concentration. It is shows that the higher concentration of CO\textsubscript{2}, with faster mortality rate and shorter the life span of the adults. Since the study of insects using both systems is still limited, the data from this preliminary study can be used as reference for future research.

**Keywords:** *Tenebrio molitor*, mortality, CO\textsubscript{2}, FACE, ORVS, life span

**ABSTRAK**

*Tenebrio molitor* L. (Coleoptera: Tenebrionidae) adalah serangga perosak simpanan gandum, di mana serangga tersebut telah digunakan sebagai subjek dalam kajian Pengurusan Perosak Bersepadu (IPM). Tujuan kajian ini adalah untuk mengenal pasti kepentingan dalam menjalankan kajian berkaitan serangga, terutama *T. molitor* di dalam Free Air CO\textsubscript{2} Enrichment (FACE) System dan Open Roof Ventilation Greenhouse System (ORVS). Sistem FACE menyediakan mikro iklim semulajadi dan interaksi-biotik, manakala ORVS ialah persekitaran buatan dimana parameter-parameter persekitarannya telah diregulasi. Perbandingan yang lebih tepat boleh dibuat daripada hasil yang diperolehi di bawah faktor-
faktor persekitaran yang sama, termasuk peningkatan kepekatan CO\textsubscript{2}. Berdasarkan hasil, kadar kematian \textit{T. molitor} dewasa di dalam ORVS (5-6 minggu) adalah terpantas, diikuti oleh FACE (9-10 minggu) dan RR sebagai kawalan (11-12 minggu). Perbezaan bererti \((p < 0.05)\) antara masa terpanjang dihitung berdasarkan individu dewasa terakhir yang mati, antara ORVS berbanding RR iaitu selama 6 minggu. Oleh itu, kadar kematian/jangka hayat \textit{T. molitor} dewasa adalah berkadar terus dengan peningkatan kepekatan CO\textsubscript{2}. Ini menunjukkan bahawa semakin tinggi kepekatan CO\textsubscript{2}, semakin cepat kadar kematian dan semakin pendek jangka hayat \textit{T. molitor} dewasa. Oleh kerana kajian terhadap serangga menggunakan kedua-dua sistem tersebut masih kurang, data-data daripada kajian awal ini boleh digunakan sebagai sumber rujukan untuk kajian-kajian pada masa hadapan.

Kata kunci: \textit{Tenebrio molitor}, kematian, CO\textsubscript{2}, FACE, ORVS, jangka hayat

INTRODUCTION

Open Roof Ventilation Greenhouse System (ORVS) is a closed system that has been created and built to provide an environment with high atmospheric CO\textsubscript{2} concentrations (Albright 2002). Other environmental factors such as inside and outside temperature and humidity were measured and controlled using psychrometers (Boulard & Draoui 1995). Generally, this system has been developed to provide a suitable environment by supplying adequate and consistent CO\textsubscript{2} concentration for plant growth and development inside the greenhouse. ORVS is a closed system with movable shade which requires computer control and a good PPFD sensor (Albright et al. 2000). According to Vanaja et al. (2006), the circular tube serves to release or disperse the CO\textsubscript{2}-enriched air and has been assisted by air blowers to ensure the CO\textsubscript{2} gas is evenly distributed within the chamber. Nowadays, a normal practice for cooling the greenhouse atmosphere is by opening the vent (Kittas et al. 1997). Because of that, it is important to minimize the CO\textsubscript{2} loss by maintaining the same level of CO\textsubscript{2} in and CO\textsubscript{2} out (Ohyama et al. 2005).

FACE is an open system which is built to provide an ambient environment with high CO\textsubscript{2} concentrations to conduct research on vegetation and other ecosystem components (Hendrey et al. 1993). The field conditions with natural microenvironment and biotic interactions become the most important factor in the construction of this system (Machacova 2010). Atmospheric CO\textsubscript{2} concentration at daytime was elevated by~130 ppm in a FACE (Miglietta et al. 2001). Pure CO\textsubscript{2} concentrations about 510 ppm has been distributed to the six CO\textsubscript{2} elevated octagons from a central CO\textsubscript{2} tank (Scherber et al. 2013). A ringshaped pipe surrounding the plot works in CO\textsubscript{2} delivery in the FACE system and is disseminated by vertically oriented pipes. CO\textsubscript{2}-enrich emissions are also controlled by the vertical pipes valves where it can be opened and closed depending on wind direction changes (von Felten et al. 2007). To increase the CO\textsubscript{2} mixing rate with air, the blowers (Pinter et al. 2000) or injecting CO\textsubscript{2} at high pressure through small orifices (Miglietta et al. 2001) were used and conducted in the FACE system.

To compare the results obtained from the ORVS treatment (closed system) with the FACE system (open system) is the significance of this study to be carried out. FACE system provides a natural microclimate such as changing weather conditions and biotic interactions among individual plants (Machacova 2010) and animals. The large-scale FACE plots also displaying the most realistic future environmental conditions due to the increment of CO\textsubscript{2} levels (Ainsworth & Long 2005). Otherwise, ORVS is an artificial environment (Kellomaki et al. 2000), where certain parameters such as humidity, CO\textsubscript{2} concentration and temperature.
has been controlled or manipulated (Albright 2002; Sanchez-Guerrero 2005), with adequate airflow rates between internal and external greenhouse environment (Harmanto 2006).

Other than that, only side-by-side tests of ORVS and FACE technology, where the studies conducted under the same enrich CO₂ levels and on the same environmental factors can be compared more accurately and reduce the bias (Ainsworth & Long 2005). According to Kimball et al. (1997), impacts of elevated CO₂ studies are often conducted using the FACE approach as it can demonstrate more accurate and definitive results. In this study, the samples of *T. molitor* were used to determine the direct effects of high CO₂ level on their development, survivability, morphology and genetics.

Then, it is important to identify the direct impacts of climate change, especially due to the increment of atmospheric CO₂ concentration towards animals and plants. Most of the previous studies that have been conducted using FACE and ORVS have focused on plants. Based on observations, there are still less publications related to the study of insects conducted in both systems, especially ORVS. In general, one of the ORVS functions is to provide a closed environment with insect screen to prevent pests from approaching crops (Kittas et al. 2005). Therefore, a study on *T. molitor* was carried out on both systems to enhance knowledge of the high CO₂ concentration effects on its morphology, biology and genetics, which subsequently became an indicator for other insects. The objective of this study is to determine the significance of *T. molitor* study in two different systems with enriched CO₂ concentrations, including FACE and ORVS.

**MATERIALS & METHODS**

**Rearing Process of Mealworm Beetle, *Tenebrio molitor***
The larvae of mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) was obtained from a local supplier located at Bandar Baru Bangi, Selangor. To ensure that the larvae are *T. molitor*, the species key by Bousquet (1990) was referred during the identification process of species based on its morphology. The larvae samples were reared in 40 × 28 × 24 cm plastic aquariums and were placed in the Cytogenetic 2 Laboratory. To ensure that the *T. molitor* larvae get enough food and water sources, oats and cucumbers have been supplied throughout the rearing activity (Siemianowska et al. 2013). Certain tools such as CO₂ Meter Version 8802-EN-00 (CO₂ concentration in ppm unit) and Hygrometer Digital (temperature (°C) and humidity (%) were used to measure specific parameters and were recorded. The samples of *T. molitor* larvae were monitored until the emergence of their adults.

**Isolation, Observation, Collection, Preservation and Analysis of *Tenebrio molitor* Adults**
Aquarium size 19 × 14 × 12 cm has been set up by 30 units. Each aquarium was filled with 4 cm height of sawdust at the bottom and 4 cm height of sifted soil. After that, forty individuals of adults were picked and put in each aquarium. Every ten sets of aquarium were placed in Free Air CO₂ Enrichment System (FACE) and Open Roof Ventilation System (ORVS) with an increment of CO₂ gas and Rearing Room (RR) as control. CO₂ concentrations (in ppm), humidity (%), temperature (°C), no of valve and wind speed (m/s) were observed and documented at every sampling time. The dead body of *T. molitor* adults were picked using forcep, stored in vile and preserved in 70% alcohol. The mortality rate/life span of adults was recorded, measured and analyzed using Microsoft Excel version 2013.
Table 1  
No of dead adults of *Tenebrio molitor* from RR (control), FACE and ORVS system

<table>
<thead>
<tr>
<th>Week</th>
<th>RR</th>
<th>FACE</th>
<th>ORVS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>441-553</td>
<td>173</td>
<td>288</td>
</tr>
<tr>
<td>3-4</td>
<td>159</td>
<td>66</td>
<td>109</td>
</tr>
<tr>
<td>5-6</td>
<td>72</td>
<td>81</td>
<td>3</td>
</tr>
<tr>
<td>7-8</td>
<td>83</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>9-10</td>
<td>17</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>11-12</td>
<td>28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CO₂= carbon dioxide; S= during sampling activity; RR= rearing room; FACE= Free Air CO₂ Enrichment system; ORVS= Open Roof Ventilation Greenhouse system.

**RESULT**

Table 1 shows the no of dead adults of *T. molitor* from both systems and control which has been collected at every sampling activity. The dead adult samples of *T. molitor* from ten sets of aquarium were collected from both systems (FACE and ORVS) and RR. As results, the mortality rate of *T. molitor* adults in ORVS (5-6 weeks) is fastest with the highest no of dead individuals in the first two weeks (288 individuals; 72 percents), compared to FACE (9-10 weeks) and RR (11-12 weeks). The significant time difference shows by the last dead adult individual; ORVS vs RR (6 weeks), FACE vs RR (2 weeks), and ORVS vs FACE (4 weeks).

**DISCUSSION**

The release of high CO₂ gas due to anthropogenic activities has increased its concentration in the atmosphere. The presence of the CO₂ gas at high concentration levels can affect the growth rate such as prolong development times (Goverde & Erhardt 2003), abundance, richness and diversity of a herbivore (Kopper & Lindroth 2003) compared with ambient CO₂ levels. There are many studies related to the effect of CO₂ concentrations, especially on insects that have been carried out. The results show that different insects, with different maturities showing different responses to different CO₂ concentrations. Based on Spratt et al. (1985), the *Trogoderma granarium* larvae which is a destructive pest of grain products, showing the reaction effect at 60% of CO₂ after 17 days at 30°C of temperature. Other than that, the adult stage shows the lowest tolerance to CO₂ than one immature life stage (Annis 1987).

Based on the results of this study (Table 1), mortality time of *T. molitor* adults in ambient conditions, with normal CO₂ concentration are slower than FACE and ORVS. Higher CO₂ concentrations that have been released on both systems of 800-950 ppm, which is 400 ppm higher than ambient levels, have been shown to accelerate the mortality time of *T. molitor* adults.

The reduction in CO₂ concentrations reading during sampling is due to the absorptions of the CO₂ gas by the plants or relatively high photosynthetic activity (Sanchez-Guerrero et al. 2005) found in both systems especially FACE, wherein CO₂ is the most important source of photosynthesis. ORVS is a closed system which able to maintain a higher CO₂ rate longer than FACE (open system). Therefore, the duration of *T. molitor* exposed to higher CO₂
concentration levels is longer and indirectly increases the CO$_2$ absorption rate through its body surface. Because of that, it has resulted in an increase in the effects of elevated CO$_2$ on it and thereby accelerates the mortality time and reduce life span of *T. molitor* adults in ORVS. In fact, the location of the FACE system itself in the forest ecosystem also affects the CO$_2$ loss rate due to the presence of large trees.

Other studies were done on *T. molitor* which focusing on the larval development and genetic changes in FACE and ORVS. It is shown that there were no significant changes on the development of larvae samples under control condition. But, slight and moderate changes were observed under FACE and ORVS with parallel changes in their genetic data (Nur Hasyimah et al. 2018). According to Nur Hasyimah and Yaakop (2018), prolonged CO$_2$ exposure towards parent and the first generation of *T. molitor* affect their development by decreased their development pattern.

According to Mbata et al. (2000), pupae of *Callosobruchus subinnotatus* Pic (Coleoptera: Bruchidae) and adults of pharate suffer from complete mortality at a slower rate after being treated by hypoxic than hypercarbic atmosphere. Under numerous elevated atmospheric CO$_2$ concentrations, the *Platynota stultana* (Lepidoptera: Tortricidae) pupae shows a different pattern of development and mortality response than under low O$_2$ atmosphere. The pupae had a greater energy scarcity under increment of CO$_2$ concentrations than under decreased O$_2$, although a similar reduction in the rate of metabolism (Zhou et al. 2001). Similar results shown by adults of *Tribolium castaneum* death is due to the depletion of triglyceride reserves caused by high CO$_2$ level (Ofuya & Reichmuth 2002).

**CONCLUSION**

FACE and ORVS have been built to assist in studies related to elevated CO$_2$ in the ecosystem. The effects of elevated CO$_2$ concentrations above the ambient level on insects, especially *T. molitor* can be compared more accurately by controlling the other abiotic factors in the environment. Besides, both FACE and ORVS conditions with enriched-CO$_2$ concentration are mimic to the expected future ecosystem that are currently experiencing an increase in CO$_2$ level in the atmosphere, where it is one of the greenhouse gases that contributes to global warming. Most of the previous studies have focused on the direct effects of atmospheric CO$_2$ enhancement on plants have been carried out on both systems. However, the use of FACE and ORVS for the study of insects is still very limited. Therefore, more studies on *T. molitor* using FACE and ORVS should be intensified to increase human understanding of elevated CO$_2$ effects towards their biology, physiology, genetics, morphology, etc. which subsequently became an indicator for other insects. As a summary, elevated CO$_2$ concentration accelerates the mortality time of *T. molitor* adults compared to ambient level (~ 450 ppm).

**ACKNOWLEDGEMENT**

This project was fully supported by Climate Change Institute (IPI) using UKM-YSD Chair in Climate Change Grant (ZF2015-025) and GUP-2016-022. Special thanks and gratitude to Universiti Kebangsaan Malaysia (UKM) and Universiti Teknologi Mara (UiTM) for providing study facilities and scholarship.
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