https://doi.org/10.17576/serangga-2024-2901-06

THE EFFECT OF ELEVATED CO_2 ON LIFE CYCLE OF *Tenebrio molitor* L. (COLEOPTERA: TENEBRIONIDAE)

Nur Hasyimah Ramli[1,2*], Hakan Bozdoğan[3] & Salmah Yaakop[1]

[1]Centre for Insect Systematics,
Department of Biological Science and Biotechnology,
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia (UKM),
43650 Bangi, Selangor, MALAYSIA
[2]School of Biology,
Faculty of Applied Sciences,
Universiti Teknologi MARA,
Cawangan Negeri Sembilan, Kampus Kuala Pilah,
72000 Kuala Pilah,
Negeri Sembilan, MALAYSIA
[3]Kırşehir Ahi Evran University,
Vocational School of Technical Sciences,
Department of Plant and Animal Production,
40100, Kırşehir, Turkey
*Corresponding author: *nurhasyimah@ns.uitm.edu.my*

Received: 14 July 2023; Accepted:14 November 2023

ABSTRACT

Tenebrio molitor L. (Coleoptera: Tenebrionidae) is a major pest of storage products and popularly used as a model species in biological research. This research is crucial in demonstrating the effects of increased CO_2 in the environment. The objective of this study was to determine the effect of life cycle of *T. molitor* (F1) at two significance systems of CO_2 namely Free Air CO_2 Enrichment System (FACE) and Open Roof Ventilation Greenhouse System (ORVS). Each stage of *T. molitor* from 10 transparent plastic containers was observed every 2 days and recorded. Prolonged of complete life cycle on the *T. molito*r in ORVS (147-172 days) observed with 43% delayed compared to FACE (71-84 days) and rearing room as control (RR) (77-105 days). The extension was due to prolong of larval to pupal development in ORVS (141-154 days), however, only 77-84 days in RR and FACE (71-84 days). The description of morphological changes in all stages and its coloration also recorded. This preliminary findings are important in predicting the *T. molitor* survivability due to climate

variability and in strategizing the Integrated Pest Management (IPM) in the warehouses of storage products.

Keywords: climate change, insect storage pest, morphology, IPM, Tenebrio molitor

ABSTRAK

Tenebrio molitor L. (Coleoptera: Tenebrionidae) ialah perosak utama produk simpanan dan popular digunakan sebagai spesies model dalam kajian biologi. Kajian ini adalah penting bagi menunjukkan kesan peningkatan CO_2 di persekitaran. Objektif kajian ini adalah untuk menentukan kesan ke atas kitaran hayat *T. molitor* (F1) didua sistem signifikan CO_2 iaitu *Free Air CO_2 Enrichment System* (FACE) dan *Open Roof Ventilation Greenhouse System* (ORVS). Setiap peringkat *T. molitor* daripada 10 bekas plastik jernih diperhatikan pada setiap dua hari dan direkodkan. Kitaran hidup berpanjangan pada *T. molitor* dalam ORVS (147-172 hari) diperhatikan dengan 43% tertunda berbanding FACE (71-84 hari) dengan bilik pemeliharaan sebagai kawalan (RR) (77-105 hari). Pelanjutan ini disebabkan oleh pemanjangan perkembangan larva kepada pupa dalam ORVS (141-154 hari), walau bagaimanapun, hanya 77-84 hari dalam RR dan FACE (71-84 hari). Perihalan dan perubahan morfologi pada setiap peringkat hidup dan pewarnaannya juga direkodkan. Penemuan awal ini penting dalam meramalkan kemandirian *T. molitor* disebabkan oleh kebolehubahan iklim dan bagi menyusun strategi Pengurusan Perosak Bersepadu (IPM) dalam gudang produk simpanan.

Kata kunci: Perubahan iklim, serangga produk simpanan, morfologi, IPM, *Tenebrio molitor*

INTRODUCTION

Infestation of insect pests may cause serious damage quantitatively and qualitatively on the storage products e.g. by *Oryzaephilus surinamensis, Tribolium castaneum, Sitophilus oryzae* and *Cadra cautella* on the rice product (Syarifah Zulaikha et. al. 2018). While, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) is a major pest that attacks a various types of grain storage and flour (Garcia et al. 2003). The *T. molitor* is often used for biological research as model species to prove the fundamental data in field of biology and easy to rear for mass rearing production which involved low cost (Adamski et al. 2019; Zanuncio et al. 2008). Besides that, the *T. molitor* species and other beetle species have a very complex life cycles and diverse due to their high adaptability and adaptation mechanism on environmental changes which depend on its stage of development (Boggs 2009).

Global climate change was influenced by greenhouse gases (GHGs) including water vapor, carbon dioxide (CO_2), methane (NH_4) and nitrous oxide (NO_2) with the second largest gas released is CO_2 (Machacova 2010). Increment of CO_2 concentrations in the atmosphere due to pre-industrial activities can affect the ability of organisms especially insects to adapt and survive (Guerenstein & Hildebrand 2008). Nur Hasyimah (2019) reported that CO_2 concentration is expected to double by the end of this century. It will indirectly affect the body mass, growth, and resistance of insect populations.

Previously, some studies were conducted to determine the effects of certain abiotic factors such as carbon dioxide (Nur Hasyimah et al. 2018a, 2018b; Nor Atikah et al. 2020), temperature and humidity (Simon et al. 2013) and variations in oxygen density (Klok et al. 2004) on morphometric and life cycle of *T. molitor*. As a result, the factors mentioned have shown impact on the development and physiology of *T. molitor*. Furthermore, the life span and

mortality rate of the *T. molitor* were monitored by refering to the recent studies done by Morales-Ramos and Rojas (2018), Park et al. (2014) and Sanchez-Guerrero et al. (2005). A recent study by Nur Hasyimah et al. (2018a) have foccused on the development only on the larval stages of the *T. molitor* at different concentration. However, no study has been conducted to investigate the effect on life cycle in relation to climate change of the *T. molitor*. Therefore, the objective of this study was to determine the impacts on life cycle of *Tenebrio molitor* (F1) in high CO_2 concentration of Free Air CO_2 Enrichment System (FACE) and Open Roof Ventilation Greenhouse System (ORVS).

MATERIALS AND METHODS

Rearing and Preparation of *Tenebrio molitor* Parent (P) Samples

The rearing process of *T. molitor* larvae was conducted prior to the experiment in both CO_2 systems in the rearing room (RR), as an control treatment. Both CO_2 systems, Open Roof Ventilation Greenhouse System (ORVS) and Free Air CO_2 Enrichment System (FACE) were set up by Climate Change Institute, Universiti Kebangsaan Malaysia (UKM). The larvae were observed every two days until the adult samples emerged. Adequate meal sources such as oats, cucumbers and carrots have been supplied for the larvae to ensure the normal development for their body size (Morales-Ramos & Rojas 2018). A total of 30 transparent plastic containers containing 4 cm height of sawdust and 4 cm height of sifted soil were set up. Then, 40 individuals of *T. molitor* (parent) were randomly inserted into each of these transparent containers with a total of 10 transparent containers were placed at ORVS, FACE and RR.

Open Roof Ventilation Greenhouse System (ORVS)

The ORVS system is a closed chamber which was developed at UKM. The pure CO_2 sprays in the system for 2 hours from 9 a.m. to 11 a.m. at a concentration of 400-950 ppm daily (Figure 1). This process was halted once the CO_2 concentration levels ranged between 800-950 ppm. In contrast to FACE, an ORVS is a closed system with a moveable canopy or open roof vent. It is controlled by a computerised system and high-quality PPFD sensors (Albright et al. 2000). Abiotic parameters such as temperature (°C), humidity (%), and CO_2 concentration (ppm) and velocity wind, have no significant effect on system performance (Boulard & Draoui 1995). Several Psychro Metres were used to monitor humidity and temperature, both inside and outside of the greenhouse. A relative temperature (0.1–0.2°C), humidity (2.4%), light intensity (>94–95% than the ambient level) were detected inside the ORVS. The ventilation process has conducted which is mostly during the day to regulate the temperature (Boulard & Draoui 1995) and also ensure that the air humidity can reduced (Harmanto et al. 2006).

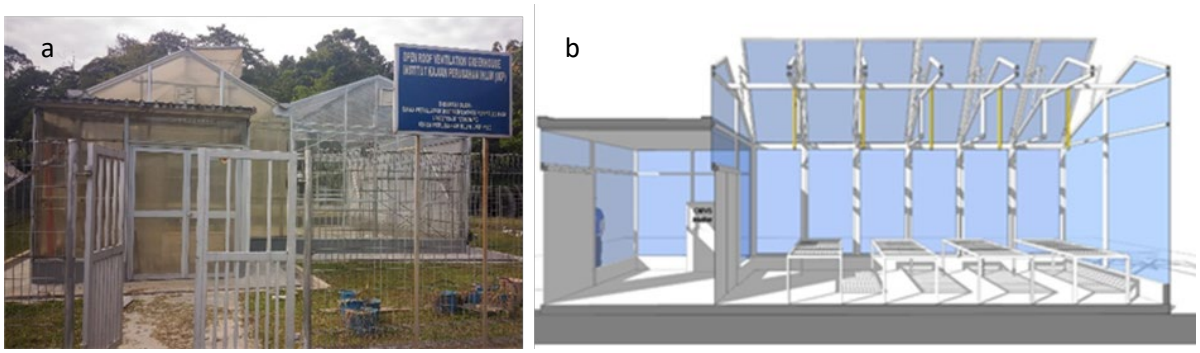

Figure 1. a) Actual; and b) prototype of Open Roof Ventilation Greenhouse System Free Air CO_2 System (FACE)

Although the FACE is an open system, it was built by maintaining its environmental parameters including temperature, humidity, velocity of wind and others because it can reduce the effects of CO_2 (Taylor et al. 2000). This system mimics natural environment except the CO_2 concentration (Figure 2a-b). The FACE system was used throughout this study to identify the effects of climate change on *T. molitor* at normal temperature (26°C-40°C) and humidity (54%-95%). However, the concentration of CO_2 is between 400-550 ppm and the original concentration released was between 800 ppm - 950 ppm and the distribution to separate pipes automatically controlled by computer systems. FACE system components include intelligent control panels, control systems (valves 1, valve 2, valve 3 and buzzer), sensors (temperature, humidity, CO_2 and wind speed), browser, internet, PC applications and phone applications. The system operates with support from Xbee wireless network sensors over four sensor nodes EZ and one wind speed sensor (WS) node. All four EZ sensor nodes are built along with temperature, humidity and CO_2 sensors while the wind speed (WS) sensor node found on the speedometer. The sensor data will be sent to the server immediately once received by the Sensor Programmable Control (SCP) using communications gsm/gprs. Android and desktop can be used to view data storage on server database (Nur Hasyimah et al. 2018b).

Figure 2. Free Air CO_2 System (FACE)

Rearing Room (RR)

The rearing room (RR) located in the Systematics Labaratory 2, Anuwar Mahmud Building, UKM was set up as a control room for observation of *T. molitor*. The RR is an enclosed space with 214 × 166 inches in size and has a tiny aperture for ventilation. The transparent plastic containers consists of the *T. molitor* samples were placed on the bench (134 × 36 × 34 inch). The room is free from insecticide and chemical contamination and suitable to be used as a rearing room. The room's environmental conditions were maintained and monitored by measuring abiotic factors like humidity (55% – 65.8%) and temperature (27.9°C – 28.8°C) for bias-free results, according to Singh (1982). The concentration of CO_2 in the RR is in range of 441–553 ppm.

Species Observation and Monitoring

First generation (F1) of Tenebrio molitor larvae and adult sampling process

Monitoring on larvae was carried out every two days. The F1 larvae sampling process starts after two weeks. A total of 40-60 individuals of *T. molitor* larvae from each system (FACE and ORVS) and RR were collected randomly using forceps from the original transparent container (19 x 14 x 12 cm). Next, the larvae sample was placed in a rectangular container (24 x 10 x 2 cm) and labeled before being taken to the laboratory. Dead adult of *T. molitor* samples (P) were collected, stored in rectangular and labelled. The larvae and adult samples were stored in the microcentrifuge tube (1 ml) and preserved using 70% alcohol.

Egg to larval stage

A total of five eggs of *T. molitor* contained in transparent plastic containers in the rearing room were collected and isolated into a rectangular plastic container. The eggs isolation was carried out using the Zeiss model microscope, Stemi D4 (Germany). The observations were carried out on the samples daily until they emerged as first instar larvae. However, the eggs samples of *T. molitor* were unable to be collected from FACE and ORVS samples due to the combination of medium (sawdust and sifted soil) with the small size of the eggs in the transparent plastic container. Other than that, high CO_2 concentration at both systems affect the ability to respirate if stay in longer period.

Larval to pupal stage

A total of 40 individuals larvae were randomly collected from each transparent plastic container at FACE, ORVS and RR every two days. Each larva from each transparent plastic container was isolated into a rectangular plastic container, measured and recorded. Then, these samples were stored in a microcentrifuge tube (1 ml) and preserved with 70% alcohol. It was assummed that the larvae in each transparent plastic container placed on FACE, ORVS and RR grow up and mature at the same time. It is due to the rearing process at both CO_2 systems and RR were prepared at the same time. These sampling processes were continued until the last individual larva was recorded. A total of 212 individuals (FACE), 420 individuals (ORVS) and 224 individuals (RR) of *T. molitor* larvae were monitored and only samples in good condition were observed to minimize bias. This is because some samples of larvae were shrink and change to black in color after being preserved in 70% alcohol solution. While for adults *T. molitor*, certain individuals were broken with a separation of body parts. The observation of the samples was conducted using Microscope Dinolite 2.0.

Pupal to adult stage

The pupae samples were observed every 2 days in each RR, FACE and FACE system. Throughout the observation and collection of samples, pupae were collected and isolated into a rectangular plastic container. Observation was carried out on the pupae until adult emergence and number of adults emerged were recorded. This process was performed until the emergence of adult of the last pupa. A total of 210, 112 and 86 pupae were recorded in FACE, ORVS and RR, respectively.

Adult stage

Each individual of adult was monitored to determine the development stage up to maturity. Monitoring results have been recorded.

Imaging and Recording Data Processes

Microsoft Excel and Word 2013 software were used to store the data. Dinolite 2.0 Microscope and DSLR Model Cannon EOS REBEL XS 1000D were used to record the images of different life stages of *T. molitor*.

RESULTS

Tenebrio molitor life cycle begins with the discovery of eggs, about 6 to 8 days after adult beetles were put in the plastic containers that has been placed in every system including FACE, ORVS and RR. It was observed that the shape of eggs is nut-shaped, white in color and found singly, not in a colony (Figure 3a). Hence, the hatching process occurs within 3 to 6 days. The larval stage represents the longest stage of development compared with the other stage. The larval development period varies at different systems (FACE, ORVS and RR) (Table 1). There are several variations of natural colors from early stages to mature which can be observed in some samples of *T. molitor* larvae (Figures 3b-d) including transparent white, milk white, yellowish orange and tanned. The earliest recorded larvae are transparent white (instar 1 and instar 2), and then it becomes darker at a more mature stage. The last instar larva observed are tanned and less active.

There were four different pupa sizes recorded (between 10 mm to 14 mm) before they turned into adult beetles, and the pupae become darker and smaller in size toward adult. Pupa transformation to adult stage takes about 5 to 14 days. In the early stages, the pupa is transparent white with larger, longer, straighter, and soft body structure than the more mature pupae. It is slowly changing to yellowish tan with a hardened and slightly curved epidermis (Figures 3e-g). Based on observations, the adult takes about 4 to 7 days for each individual to reach maturity after hatched. In the early stages of hatching, the beetle is less active until its wings are dry and not folded. The observations on adults *T. molitor* shows that there are some color changes shown from whitish to shiny black without involving size changes. Some of the color recorded are white, yellowish white, brown, dark brown and black glossy (Figures 3h-k). Figure belows show the egg, larvae, pupae and adult (Figures a-k) stages of *T. molitor* in different CO_2 systems.

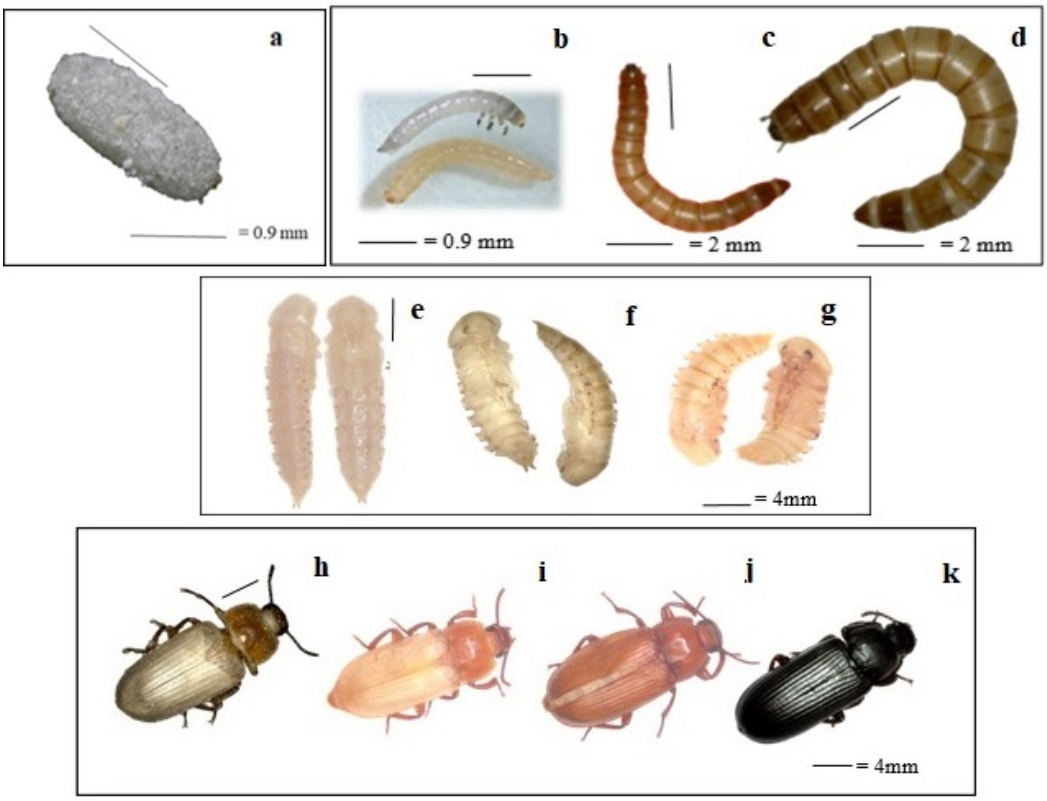

Figure 3. a, Egg; b-d, different instar*;* e-g, interior and exterior parts of pupa at different levels of maturity; h-k, color changes of adult *Tenebrio molitor*

Table 1 shows the life span of *T. molitor* after being exposed to high CO_2 concentrations in FACE and ORVS, while RR acts as control. The observations on *T. molitor* eggs show that the duration for egg to hatch was 3-6 days in both CO_2 systems and RR. Due to several factors, the developmental time from the egg to the larval stages in the FACE system and ORVS was somewhere equal to the RR. The mean of time taken from larva to pupa stage in RR (77-84 days) and FACE (71-84 days). However, longer periods of growth between larva to pupa were observed in *T. molitor* in ORVS (141-154 days). The time taken from pupa emergence to adult stage in each system also in a similar range of 3-15 days. Both systems with CO_2 treatment show the earliest individual of pupa formed after 3 days compared to RR (5 days).

Overall development life cycle of *T. molitor* were 85-103 days and 77-105 days in RR and FACE, respectively. However, in FACE shows the earliest developmental changes between larva to pupa (day 71) and pupa to adult (day 3). In fact, the earliest individual who completed its life cycle was recorded at FACE (day 77). It was different about eight days (RR) and 70 days (ORVS) compared to the other two systems. However, longer duration is required by individual *T. molitor* at ORVS (147-172 days) to complete their life cycle from egg to adult stage compared to FACE and RR.

Table 1. Life Cycle of *Tenebrio molitor* at RR (Control), FACE and ORVS (CO_2 treatment) Systems

System/Level	Egg to Larva (Day)	Larva to Pupa (Day)	Pupa to Adult (Day)	Total (Day)
RR	3-6	77-84	5-14	85-103
FACE	3-6	71-84	3-15	77-105
ORVS	3-6	141-154	3-12	147-172

*ORVS = Open Roof Ventilation Greenhouse System; FACE = Free Air CO_2 Enrichment System; RR = Rearing Room.

DISCUSSION

Based on the results of this study, there was no effect of CO_2 increment on *T. molitor* morphology, but only the normal changes in through-their development of body size which was supported by Park et al. (2014). During the eggs collection process, the samples are found singly. According to Ghaly and Alkoaik (2009), samples of *T. molitor* eggs were found singly and in a group with a range of 1.7-1.8 mm (length) and 0.6-0.7 mm (wide). The USDA (2016) states that the process of egg-laying *T. molitor* with nut-shaped occurs within two weeks before the white larva are found. The statement is parallel with the results of this study. The size of the eggs that are too small (appx. 1.8 mm) is a constraint to be found in the medium consists of sifting soil and sawdust in the transparent plastic container.

The *T. molitor* embryo development can be measured from the molting process (Park et al. 2014), about eight to 20 times (Connat et al. 1991). Mostly, *T. molitor* larvae only undergo an instar exchange process of 15 to 17 times only. The instar exchange was associated with the color difference of the *T. molitor* larvae through comparison with Park et al. (2014). However, monitoring of the instar larva changes cannot be performed directly in this study due to several factors including those samples unable to survive after being isolated into non-medium plastic containers, in FACE and ORVS. Based on a previous study, the first two weeks were known as incubation period (7-8 days), which is the process of fertilisation and laying up until the larva instar 1 (3-4 days). It also coincides with this study, with instar 1 larvae found in every transparent plastic container in FACE, ORVS and RR after 14 days of experiment. However, the larvae growth period varies between ORVS, FACE and rearing room (Table 1)

For *T. molitor* development time recorded to complete their life cycle involving four levels including eggs, larva, pupa and adult is at the same range in RR (85-103 days) and FACE (77-105 days). However, there were *T. molitor* individuals which transform from larval to pupal stage at fastest rate in FACE (day 71), equivalent to six days earlier than RR (day 77). Similar results are also shown between the changes of pupa into adult, equivalent to two days earlier in FACE than RR. The main factor contributing to the reduction of CO_2 concentration effects toward FACE F1 samples was probably due to instability of the FACE system (Miglietta et al. 2001).

Tenebrio molitor eggs were hatched after 4-17 days of fertilization with the development of the embryo ending after 4-6 days (Siemianowska et al. 2013). It is coinciding with the observations carried out in this study, the first instar larvae were discovered from each transparent plastic container placed on each system after 14 days. The development time from pupa to adult was also in range with previous studies of 5-6 days (Siemianowska et al. 2013), nine days (Quennedey & Quennedey 1993) and 7-9 days (Hill 2002). Based on Smith et al. (2013), the length of time taken by *T. molitor* to complete the metamorphosis process is as

follows; the incubation process (4-19 days), the larva stage (70 days) and the pupa to adult (6-18 days) and it shows the similarity with the life span of *T. molitor* of RR and FACE systems. The *T. molitor* life span in ORVS was different than FACE and RR. At a high CO_2 concentration of 300-950 ppm, to complete life cycle of *T. molitor* was longer, especially at larval stage longer that took maximum of 154 days, and automatically prolong their life span. The CO_2 concentrations in ORVS were regulated to match estimated CO_2 concentration in the atmosphere in 2100 ranging from 540 to 970 ppm (Cornelissen 2011). The duration differences in the *T. molitor* life cycle at ORVS differ significantly compared to the FACE and RR systems of 70 days, extending the life cycle to 147-172 days. These results are in parallel with Goverde and Erhardt (2003), which is an increase in CO_2 concentrations, extending the development of *Coenonympha pamphilus* (L.) (Lepidoptera: Satyridae) cultivated in four species of home plants.

Contrary to statements by Kim et al. (2015), the time taken to complete a larval stage in normal condition is 160 days. It differs from the results in rearing room (RR) in this study with only 77-84 days, but it is almost equal to the development duration of ORVS larvae that has been treated with high CO_2 concentrations. A study by Boggs (2009) suggests that the time taken by the larva to complete its stage of development is between 45-60 days.The results shown by our ORVS treatment disagree to Brooks (1957) where the time required by larva and pupa of *Leis axyridis* to complete their life cycle is significantly shorter. Morales-Ramos et al. (2010) also states that the development period of *T. molitor* larvae is positively correlated with the number of instar and it is likely to be influenced by biotic and abiotic factors in the environment including temperature, humidity, photocatalyst, oxygen concentration and population density.

However, the prolong life cycle from egg to adult level also affects the development, physiology, behaviour and reproduction of *T. molitor*. This is because the longer the growth period at the larva level will increase its survival rate, increase the rate of food intake versus generation, increase digestion, lower conversion efficiency and absorption of food in the body of four generations of *Archaea janata* (L.) (Lepidoptera: Nuctuoidea) sequentially in high concentrations of CO_2 (Rao et al. 2013). According to Karowe (2007), larvae of *Colias philodice* (Lepidoptera: Pieridae) that ate in high CO_2 environments have lowered their ability to digest food. It affects the rate of growth, instar period and weight of the pupa.

Ventilation process should be conducted in greenhouses especially during the day to control and maintain the temperature (Boulard & Draoui 1995). To control high humidity (>85%), 25% roof was opened to reduce the humidity rate (Harmanto et al. 2006; Sanchez-Guerrero et al. 2005). While in FACE, the system has been built by maintaining environmental parameters including temperature, humidity, wind velocity, etc. that can lower the CO_2 effect and reduce the bias (Ainsworth & Long 2005). Hence, it proves that the difference in the development time required by *T. molitor* to complete the metamorphosis process, is particularly influenced by high CO_2 concentrations in the atmosphere. It involves elongation or prolong of its life cycle, as shown by *T. molitor* at ORVS.

These results indirectly indicate that the *T. molitor* species is able to adapt or be susceptible to high CO_2 concentrations in the atmosphere from the egg level until emerge as adult. German cockroach growth rate, *Blattella germanica* (L.) (Orthoptera: Blattidae) was monitored after being exposed to high concentrations of CO_2 for 3 minutes each week until reach maturity indicates the time elongation required to complete the live cycle through the molting process for about 14% -53% compared to control samples (Brooks 1957). According

to Conner (1988), a short period of growth is aimed at lowering the death risk of the *Bolitotherus cornutus* (Coleoptera: Tenebrionidae) larvae which eventually resulting in the production of small adult sizes and fix the growth of larvae.

Based on our observation, pupa body surface was chewy, soft and it gets hardened with its shortening size. Normally, the larva will move on the surface of the medium before turning into a pupa. It takes about 5-6 days at optimum temperature (Siemianowska et al. 2013). Changes of pupa to adult stage involve changes in the body shape as a whole. It also involves the change of body color through its maturity level. The similarity that can be seen between the larva, pupa and adult was on their body color at the beginning of its development stage where the color of the larva, pupa and adults are white. Similar results were also recorded by Ghaly and Alkoaik (2009), Park et al. (2014) and Siemianowska et at. (2013). Therefore, the life cycle of *T. molitor* at high CO_2 concentration is the same in the natural environment. This proves that the increase in CO_2 concentration does not affect the life cycle of *T. molitor* as a whole. However, Zeuss et al. (2014) stated that butterflies will change their color to dark-coloured in cooler climate, while lighter at warmer climates.

CONCLUSION

As a conclusion, the increase in CO_2 concentration in the environment above ambient level is able to change the life cycle of *T. molitor* after prolong exposure. The life cycle of *T. molitor,* involves extending the time period for the change from larval to pupal stage in ORVS. It has indirectly resulted in the expansion of the life cycle of *T. molitor* as a whole, while also demonstrating that the accumulation of greenhouse gases in the environment, especially CO_2, will affect the life cycle of insects, particularly *T. molitor*. Furthermore, the data obtained from this study is a new finding on the effects of high CO_2 towards Tenebrionidae, especially *T. molitor*. Indirectly, the data also can be used as a source of reference or model to other insect species.

ACKNOWLEDGEMENTS

Special appreciation to Universiti Kebangsaan Malaysia (UKM) and Universiti Teknologi Mara (UiTM) for providing study facilities and scholarship.

AUTHORS DECLARATIONS

Funding Statement
The authors acknowledged the financial support from the Climate Change Institute (IPI) grant, with grant number ZF-2019-005 and GUP-2018-037.

Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue required for this research.

Data Availability Statement
Not relevant

Authors' Contributions

Nur Hasyimah Ramli (NHR) conceived this research, designed and conducted the experiments; Salmah Yaakop (SY) as a consulting expert to ensure that the experiment is carried out well and reviewed the writing of the manuscript, and Nur Hasyimah Ramli and Salmah Yaakop wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript.

REFERENCES

Adamski, Z., Bufo, S. A., Chowański, S., Falabella, P., Lubawy, J., Marciniak, P., Pacholska, B.J., Salvia, R., Scrano, L., Słocińska, M., Spochacz, M., Szymczak, M., Urbański, A., Walkowiak-Nowicka, K., & Rosiński, G. (2019). Beetles as model organisms in physiological, biomedical and environmental studies - A review. *Frontiers in Physiology* 10(3): 1–22.

Ainsworth, E.A. & Long, S.P. 2005. What have we learned from 15 years of Free Air CO_2 Enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO_2. *New Phytologist* 165: 351–372.

Albright, L., Both, A.J. & Chiu, A.J. 2000. Controlling greenhouse light to a consistent daily integral. *Transactions of the ASAE* 43(2): 421–431.

Boggs, C.L. 2009. Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology* 2: 27–37.

Boulard, T. & Draoui, B. 1995. Natural ventilation of a greenhouse with continous roof vents: measurements and data *analysis. Journal of Agricultural Engineering Research* 61: 27–36.

Brooks, M.A. 1957. Growth-retarding effect of carbon-dioxide anaesthesia on the German cockroach. *Journal of Insect Physiology* 1: 76–84.

Connat, J.L., Delbecque, J.P., Glitho, I. & Delachambre, J. 1991. The onset of metamorphosis in *Tenebrio molitor* larvae (Insecta, Coleoptera) under grouped, isolated and starved conditions. *Journal of Insect Physiology* 37: 653–662.

Conner, J. 1988. Field Measurements of natural and sexual selection in the Fungus Beetle, *Bolitotherus cornutus. Evolution* 42: 736–749.

Cornelissen, T. 2011. Climate change and its effects on terrestrial insects and herbivory patterns. *Neotropical Entomology* 40: 155–163.

Garcia, M., Sosa, M.E., Donadel, O.J., Giordano, O.S. & Tonn, C.E. 2003. Effects of some sesquiterpenes on the stored-product insect *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Revista De la Sociedad Entomologica Argentina* 62: 1726.

Ghaly, A.E. & Alkoaik, F.N. 2009. The yellow mealworm as a novel source of protein. *American Journal of Agricultural and Biological Sciences* 4: 319–331.

Goverde, M. & Erhardt, A. 2003. Effects of elevated CO_2 on development and larval food-plant preference in the butterfly *Coenonympha pamphilus* (Lepidoptera: Satyridae). *Global Change Biology* 9: 74–83.

Guerenstein, P.G. & Hildebrand, J.G. 2008. Roles and effects of environmental carbon dioxide in insect life. *Annual review of Entomology* 53: 161–178.

Harmanto, Tantau, H.J. & Salokhe, V.M. 2006. Influence of insect screens with different mesh sizes on ventilation rate and microclimate of greenhouses in the humid tropics. *International Journal of Agricultural Engineering* 8: 1–18.

Heinrich, E.C., Farzin, M., Klok, C.J. & Harrison, J.F. 2011. The effect of developmental stage on the sensitivity of cell and body size to hypoxia in *Drosophila melanogaster*. *Journal of Experimental Biology* 214: 1419-1427.

Hill, D.S. 2002. *Pests of Stored Foodstuffs and Their Contro*l. Netherlands: Kluwer Academic Publishers.

Karowe, D.N. 2007. Are legume-feeding herbivores buffered against direct effects of elevated carbon dioxide on host plants? a test with the sulfur butterfly, *Colias philodice*. *Global Change Biology* 13: 2045–2051.

Kim, S.Y., Park, J.B., Lee, J.B., Yoon, H.J., Lee, K.Y. & Jim, N.J. 2015. Growth characteristics of mealworm *Tenebrio molitor*. *Journal Sericultural Entomological Science* 53(1): 1–5.

Klok, C.J., Sinclair, B.J. & Chown, S.L. 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. *Journal of Experimental Biology* 207: 2361-2370.

Machacova, T. 2010. Open top chamber and Free Air CO_2 Enrichment - approaches to investigate tree responses to elevated CO_2. *Iforest* 3: 102–105.

Miglietta, F., Peressotti, A., Vaccari, F.P., Zaldei, A., De Angelis, P. & Scarascia-Mugnozza, G. 2001. Free Air CO_2 Enrichment (FACE) of a poplar plantation: The POPFACE fumigation system. *New Phytologist* 150(2): 465–47.

Morales-Ramos, J.A. & Rojas, M.G. 2018. Effect of larval density on food utilization efficiency of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Ecology and Behavior* 108: 2259–2267.

Morales-Ramos, J.A., Rojas, M.G., Shapiro-Ilan, D.I. & Tedders, W.L. 2010. Developmental plasticity in *Tenebrio molitor* (Coleoptera: Tenebrionidae): analysis of instar variation in number and development time under different diets. *Journal of Entomological Science* 45: 75–90.

Nor Atikah, A.R., Halim, M., Nur Hasyimah, R. & Yaakop, S. 2020. Evaluation of Colour Changes, Survival Rate and Life Span of the Confused Sap Beetle (*Carpophilus mutilatus*) (Coleoptera: Nitidulidae) in Different Concentrations of Carbon Dioxide (CO_2). *Applied Ecology and Environmental Research* 18(5): 6443-6455.

Nur Hasyimah, R. 2019. Kesan peningkatan kepekatan karbon dioksida (CO_2) ke atas morfologi, biologi dan genetik *Tenebrio molitor*. PhD Thesis, Universiti Kebangsaan Malaysia.

Nur Hasyimah, R., Nor Atikah, A.R. & Yaakop, S. 2018a. The significance of free air CO_2 enrichment and open roof ventilation greenhouse systems in a study of mealworm beetle, (Coleoptera: Tenebrionidae). *Serangga* 23(2):122-129.

Nur Hasyimah, R., Nor Atikah, A.R., Halim, M., Muhaimin, A.M.D., Nizam, M.S., Hanafiah, M.M. & Yaakop, S. 2018b. CO_2 effects on larval development and genetics of mealworm beetle, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) in two different CO_2 systems. *Applied Ecology and Environmental Research* 16(2): 1749–176.

Park, J.B. Choi, W.H., Kim, S.H., Jin, H.J., Han, Y.S., Lee, Y.S. & Kim, N.J. 2014. Developmental characteristics of *Tenebrio molitor* larvae (Coleoptera: Tenebrionidae) in different instars. *International Journal of Industrial Entomology* 28: 5–9.

Rao, M.S., Srinivas, K., Vanaja, M., Manimanjari, D., Rao, C.A.R. & Venkateswarlu, B. 2013. Response of multiple generations of semilooper, *Achaea janata* feeding on castor to elevated CO_2. *Journal of Environmental Biology* 34: 747–754.

Quennedey, A. & Quennedey, B. 1993. The precocious commitment of wing anlagen in *Tenebrio molitor* revealed by the addition of 20-hydroxyecdysone. *Tissue and Cell* 25(2): 219-236.

Sanchez-Guerrero, M.C., Lorenzo, P., Medrano, E., Castilla, N., Soriano, T. & Baille, A. 2005. Effect of variable CO_2 enrichment on greenhouse production in mild winter climates. *Agricultural and Forest Meteorology* 132: 244–252.

Siemianowska, E., Kosewska, A., Aljewicz, M., Skibniewska, K.A., Polak-Juszczak, A., Jarocki, A. & Jędras, M. 2013. Larvae of mealworm (*Tenebrio molitor* L.) as European novel food. *Agricultural Science* 4(6): 287–291.

Simon, E., Baranyai, E., Braun, M., Fábián, I. & Tóthmérész, B. 2013. Elemental concentration in mealworm beetle (*Tenebrio molitor* L.) during metamorphosis. *Biological Trace Element Research* 154: 81–87.

Singh, P. 1982. The rearing of beneficial insects. *New Zealand Entomologist* 7(3): 304–310.

Smith, M.A., Fernandez-Triana, J. L., Eveleigh, E., Gomez, J., Guclu, C., Hallwachs, W., Hebert, P.D.N., Hrcek, J., Huber, J.T., Janzen, D., Mason, P.G., Miller, S., Quicke, D.L. J., Rodriguez, J.J., Rougerie, R., Shaw, M.R., Varkonyi, G., Ward, D.F., Whitfield, J.B. & Zaldivar-Riveron, A. 2013. DNA barcoding and the taxonomy of Microgastrinae wasps (Hymenoptera, Braconidae): Impacts after 8 years and nearly 20000 sequences. *Molecular Ecology Resources* 13: 168-176.

Syarifah Zulaikha, S. A., Halim, M., Nor Atikah A.R. & Yaakop, S. 2018. Diversity and abundance of storage pest in rice warehouses in Klang, Selangor, Malaysia. *Serangga,* 23 (1): 89-98.

Taylor, C.J., Young, P.C., Chotai, A., Mcleod, A. & Glasock, A.R. 2000. Modelling and control design for Free Air Carbon Dioxide Enrichment (FACE) Systems. *Journal of Agricultural and Engineering Research* 75: 365–374.

USDA. 2016. Stored Grain Insect Reference. Washington: United States Department of Agriculture.

Zanuncio, J.C., Pereira, F.F., Jacques, G.C., Tavares, M.T. & Serrão, J.E. 2008. *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae), a new alternative host to rear the pupae parasitoid *Palmistichus elaeisis* Delvare and LaSalle (Hymenoptera: Eulophidae). *The Coleopterists Bulletin* 62(1): 62–66.

Zeuss, D., Brandl, R., Brändle, M., Rahbek, C. & Brunzel, S. 2014. Global warming favours light-coloured insects in Europe. *Nature Communications* 5: 3874.