Vol. 1 No. 1 (2020)

Published: 2020-03-20

https://www.ijcbs.org/index.php/ijcbs/article/view/3

Anoxia as a treatment against *Tetranychus urticae* and *Spodoptera littoralis*

Rania Ahmed Abd El-Wahab
Plant Protection Research Institute, Agriculture Research Center, EGYPT
rania-proline@hotmail.com

ABSTRACT

The created anoxic environment has inferred its effect against specific agricultural pests as *Tetranychus urticae* and *Spodoptera littoralis*. It was depending on the exposure of both pests with certain stages to 0 ppm O_2 and 5000 ppm CO_2. After anoxia exposure for 8h, no hatchability was detected of both pests' eggs. Consequently, hatchability percentages were 78.07% and 64.11% for *T.urticae* and *S.littoralis*, respectively, after anoxia exposure for 2h. While exposure to 4h to anoxic conditions resulted in a reduction of hatchability percentages which recorded 32.01% and 20.78% for the same arrangement, respectively. Then anoxia effect expanded on the survival percentages of the resulted larvae of *T.urticae* and *S.littoralis*, they were 20.36% and 31.78%, respectively, for 2h. While exposure to 4h to anoxic conditions resulted in a decrease in survival percentages which recorded 5.05% and 5.41% for the same arrangement, respectively. Estimated values of LT_{50} were 5.7,4.1 and 4.77 h, respectively, for eggs, adult males and females of *T.urticae*, respectively. While, LT50s were 6.09,3.15,4.21, and 6.15 h for anoxia effect on eggs, 2^{nd} and 4^{th} larval stages and pupal stage of *S. littoralis*, respectively. Anoxic stress led to oxidative stress development. Excessive reactive oxygen species translated in the significant reduction of formation in reactive oxygen scavengers (ROS) in anoxia treatments with LT50 of both pests than control. Ascorbate Peroxidase (APX) levels before exposure to anoxia were higher than after treatments. To recapitulate, increased anoxic stress is able to control agricultural pests efficiently pro rata with elevated reactive oxygen species.

Keywords: Anoxia; CO_2; Reactive Oxygen Species; *Spodoptera; Tetranychus*

INTRODUCTION

Modified atmospheres (MAs) are extending out utilized recently to manage agricultural pests even to kill or deterrent them. It might be occurred through hypoxia by low O2, and elevated levels of CO2 (Seki and Murai 2012a, b). While anoxia by zero O2 and 100% of CO2 have an anesthetic action that led to death. The mode of action of the elevated concentration of CO2 on insects was mainly around inhibition of bioelectric responses of their nervous system (Nicolas and Sillans, 1989).

Besides, many studies are associated with depletion in O2 and/or with an elevation of CO2 through modified or controlled atmospheres (MAs or CAs) which provided an eco-friendly and cost-efficient treatment against pests in stored products (Golestan and Rahimi, 2017).

On the other hand, MAs played an important role in transplanting seedlings which sullied with certain pests regularly incites their outbreak in nurseries, where specific numbers of natural enemies are restricted (Van Lenteren, 2000). To acquire seedlings free of pests and also post-harvest products, chemical fumigation with an expansive range pesticide, methyl bromide, was a regularly utilized strategy (Ristaino and Thomas, 1997; Mitcham et al., 2006). Nonetheless, methyl bromide is a noteworthy depleting substance of the ozone (Montzka and Reimann, 2011); it is likewise profoundly poisonous to people.

Nonetheless, modified or controlled atmospheres (MAs or CAs) would be an alternative of common pesticides against herbivores as spider mites (Suzuki et al.,2015).
Subsequently, the focus of this study was around the potential role of anoxia with 0% O2 and a high concentration of CO2 in controlled atmospheres (CAs) to control *Tetranychus urticae* and *Spodoptera littoralis*.

MATERIALS AND METHODS

- *Tetranychus urticae* culture

T.urticae were collected from normally infested castor oil plants (*Ricinus communis* L.). Maintenance of *T.urticae* was done under laboratory conditions (25±2˚C, and 60±5%RH) at Plant Protection Research Institute, Mansoura branch, Egypt. Colonies were reared on cleaned castor oil leaves and placed on moist cotton wool pad in Petri-dishes as indicated by Dittrich (1962), for a half year before treatments. Spider mites were transferred to the leaves by the aid of the fine camel's hairbrush. Adding water was done twice daily to prevent escaping of *T.urticae* individuals.

- *Spodoptera littoralis* culture

Spodoptera littoralis was cultured on leaves of the castor oil plant (*Ricinus communis* L.) in Plant Protection Research Institute, Agriculture Research Center, Mansoura Branch, Egypt. Larvae were

kept at 25±1 °C, 70% RH and 12L: 12D of photoperiod. Eggs, second and fourth larval instars, and pupae were used in this experiment.

-Anoxia test of tested pests

Co2 was determined by EZO-CO2™ that composed mainly of embedded NDIR CO_2 sensors able to detect 0-10000 ppm (Atlas Scientific LLC, New York, USA). It was compatible with Arduino system that was used to control the full operations and attached to the computer. Gaseous CO_2 was adapted at 5000ppm concentration with no O_2 (0 ppm). Increased CO_2 was increased gradually by the addition of calcium carbonate and hydraulic acid as the following equation:

$$HCl+CaCO3 \longrightarrow CaCl_2+H_2O+CO_2$$

Samples were placed in closed glass cube containers with almost 0.25m^3 which contained at the bottom of it added 250 ml of each chemical, then the above layer was polycarbonate set with Petri dishes and each exposed stage of certain pests. Then, sensors of CO2 were inserted from the top cover of that small chamber inside the space of it. Exposure was done for different duration of test 2,4, and 8 h beside control (0 h). Then all pests were kept in the laboratory circumstances (25±5 ºC, 75%RH). Exposure of each pest was by kept 90 individuals of each tested pest stage under the anoxia treatment which triple replicated. *T.urticae* were transferred to discs of castor oil plants which placed on moist cotton wool pad in Petri-dishes as indicated by Dittrich (1962). Concerning exposed stages of *S. littoralis*, they were placed in Petri-dishes with discs of castor oil for each required duration under anoxic conditions. Then all kept under laboratory conditions to calculate percentages of hatchability of eggs and survival of larvae. Also, LT50s of tested stages were determined by probit analysis.

-Antioxidant of enzyme activities in treated pests exposed to anoxia

APX activity was measured through the estimation of the rate of ascorbate oxidation. Each 3 mL reaction mixture was consisted of 0.1 mM H2O2+50mM phosphate buffer (pH 7) + 0.1 mM EDTA+0.5 mM sodium ascorbate, and an enzyme extract. The change of the absorbance was monitored at 290 nm and enzyme activity was expressed as the unit's min/mg protein (Nakano and Asada, 1981).

-Data Analysis

IBM SPSS was used to analyze data gained by anoxia treatments. Paired Samples correlations test to show the significant differences between anoxic treatments of exposed certain stages of tested pests. Besides, both Friedman's two-way analysis of variance by ranks that showed a highly significant difference at 1% and Cronbach's Alpha through Reliability Statistics at 5% were used.

RESULTS AND DISCUSSION

-Eggs hatchability and larval survival

Concerning the effect of certain durations of anoxia on the hatchability of the eggs of *T.urticae* and then survival of larvae was shown in the figure (1). After anoxia exposure for 8h, no hatchability occurred while control recorded the highest hatchability percentage of 95.24%.

Subsequently, hatchability percentages were recorded 78.07% and 32.01% by exposure of eggs under anoxia durations for 2 h and 4 h, respectively. Consequently, the effect of certain durations of anoxia on the hatchability of the eggs of *S. littoralis* and then survival of larvae was shown in figure (1). After anoxia exposure for 8h, no hatchability occurred while control recorded the highest hatchability percentage of 97.01%. Therefore, hatchability percentages were recorded 64.11% and 20.78% by exposure of eggs under anoxia durations for 2 h and 4 h, respectively.

Regarding the effect of specific durations of anoxia on the resulted larvae and then survival of them, data was recorded at the figure (1). After anoxia exposure for 8h, no survival occurred while control recorded the highest survival percentage of 97.21%. Subsequently, survival percentages were recorded 20.36% and 5.05% by exposure of eggs under anoxia durations for 2 h and 4 h, respectively. Consequently, after anoxia exposure for 8h, no survival occurred of *S. littoralis* larvae while control recorded the highest percentage of 96.35%. Therefore, survival percentages were recorded 31.78% and 5.41% by exposure of larvae under anoxia durations for 2 h and 4 h, respectively.

Paired Samples correlations test showed the highest significant between anoxia duration and *T.urticae* hatchability at 1% (.950**), followed by the relation between anoxia duration and *S. littoralis* hatchability at 5% (.933*). In the same trend, the Paired Samples correlations test showed the highest significant between anoxia duration and *T.urticae* survival at 1% (.840**), followed by the relation between anoxia duration and *S. littoralis* survival (.751*).

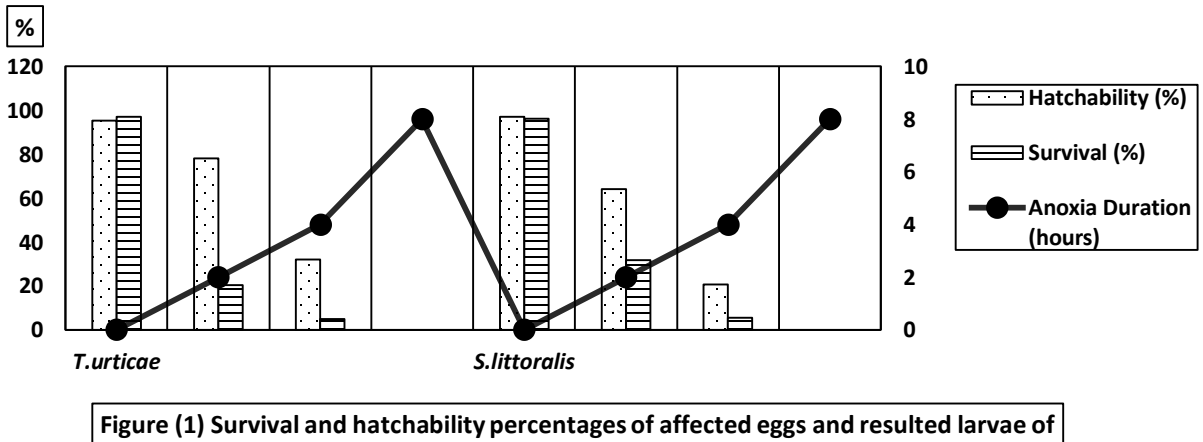

Figure (1) Survival and hatchability percentages of affected eggs and resulted larvae of both *Tetranychus urticae* and *Spodoptera littoralis*

-LT50s of Anoxia Treatments

Anoxic action against both *T.urticae* and *S. littoralis* was tested and LT50s were recorded as shown in Figure (2). LT50s were 5.75,4.1, and 4.77 h for anoxia effect of eggs, adult males and females of *T.urticae*, respectively. While, LT50s were 6.09,3.15,4.21, and 6.15 h for anoxia effect of eggs, 2nd and 4th larval stages and pupal stage of *S. littoralis*, respectively.

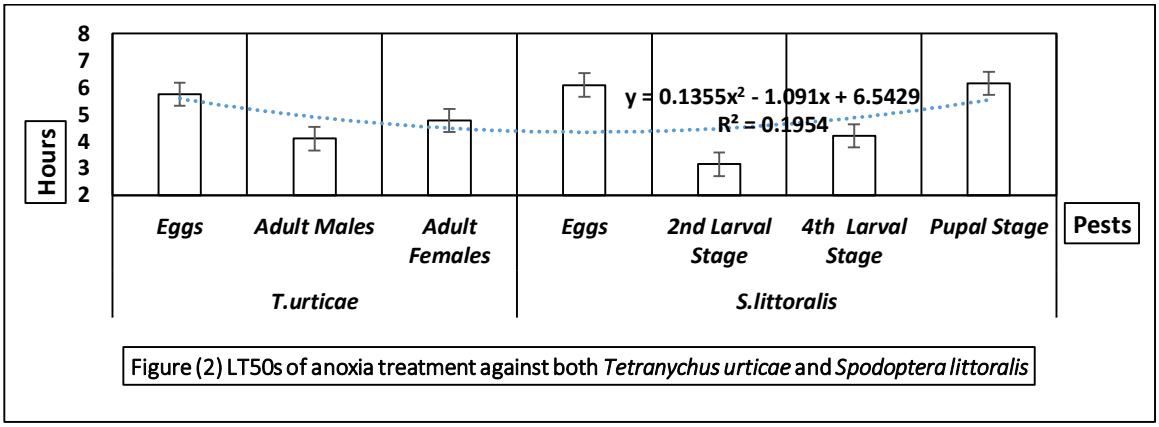

Figure (2) LT50s of anoxia treatment against both *Tetranychus urticae* and *Spodoptera littoralis*

-ROS affected by anoxia LT50s

Reactive Oxygen Scavengers (ROS) in anoxia treatments with LT50 of both *T.urticae* and *S. littoralis*, were significantly lower decrease than control ($P < 0.05$). Figure (3) showed that Ascorbate Peroxidase (APX) before exposure to anoxia was higher than after treatments. Anoxia LT50s of *T.urticae* adult males and females caused a reduction of APX with 36.37 and 35.62 %,resp. Otherwise, APX values of *S. littoralis* with its 4th larval instar and pupal stage were affected by anoxia LT50s almost higher than adults of *T.urticae*. APX recorded reduction with 41.39 and 37.99%, resp., for both tested stages as mentioned previously. Friedman's two-way analysis of variance by ranks showed a highly significant difference at 1% (.01**) in all treatments of APX ratios before and after exposure to anoxia. Also, paired-samples correlations between treatments before and after anoxia showed the highest significant correlation at 1% (.982**). Cronbach's Alpha through Reliability Statistics showed a significant difference at 5% in the decrease of APX between males and females of *T.urticae* =.750* and for stages of *S. littoralis*= .631.

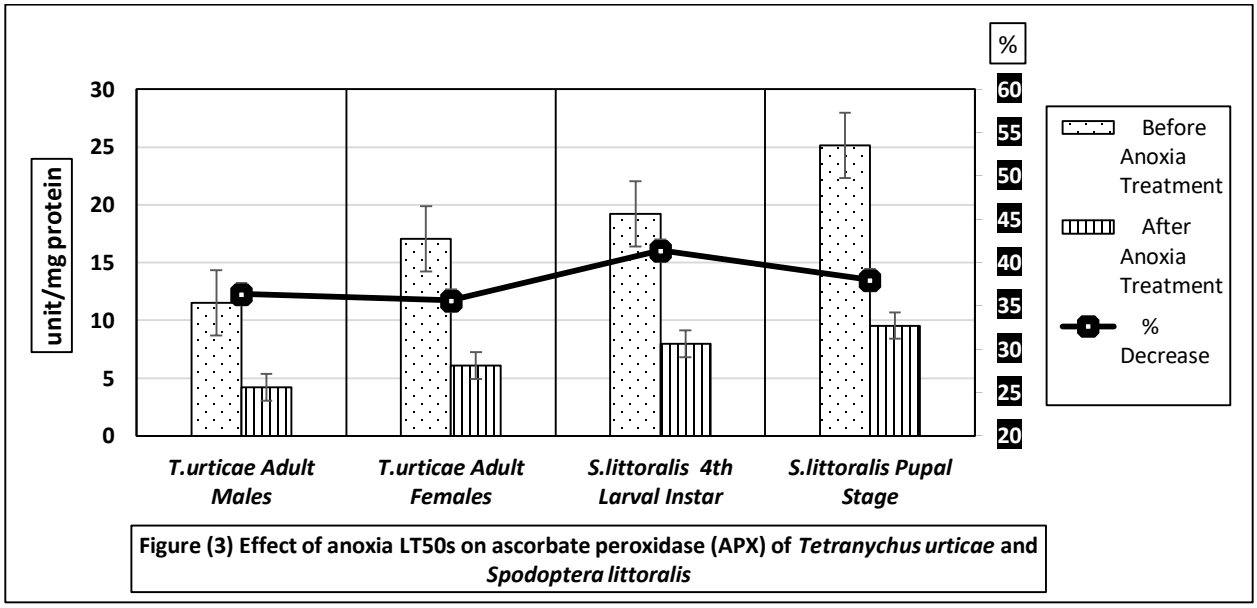

Figure (3) Effect of anoxia LT50s on ascorbate peroxidase (APX) of *Tetranychus urticae* and *Spodoptera littoralis*

%Decrease = ROS (APX) ratio after anoxia of the exposed pest / ROS (APX) ratio before anoxia of the exposed pest *100

DISCUSSION

Numerous insects, however not all, for example, larvae of the fruit fly (Callier et al., 2015), enter a comatose state of the neuromuscular when presented to anoxia (Rodgers et al., 2010) and longer anoxic exposures decrease endurance and increment the time it takes for insects to recuperate from anoxia (Lighton and Schilman, 2007).

Anoxic mortality possibly happens when ATP levels are near zero and anoxic endurance may consequently include some capacity to manage/keep up ATP at a low yet non-zero level (Campbell et al., 2018). The low ATP levels are probably going to lessen the action of particle intention ATPases, bringing circa a steady loss of particle homeostasis during anoxia. This is especially dangerous if extracellular K+ concentration rises (Rodgers et al., 2010; Campbell et al., 2018), as a disturbed transmembrane [K+] inclination prompts cell layer depolarization, which can start apoptotic or necrotic procedures (Hochachka, 1986; Armstrong et al., 2011; Bayley et al., 2018). Raised extracellular [K+] has been seen in both cerebrum (Rodriguez and Robertson, 2012) and hemolymph (Campbell et al., 2018) of anoxic D. melanogaster. Likely, anaerobic metabolism caused serious acidification as detected in *D. melanogaster* hemolymph. The anoxic injury has then resulted from anaerobic acidosis (Storey and Storey, 1990) and it is additionally conceivable that injury is connected to oxidative damage, especially during re-oxygenation upon recuperation from anoxia (Hermes-Lima et al., 2015; Moreira et al., 2016).

Moreover, ATP depletion with its downstream effects could also participate in anoxic injury as proteins damage, osmotic homeostasis disruption beside formation of excessive reactive oxygen species (ROS) during re-oxygenation (Harrison et al.,2018; Bergamini et al.,2004; Hermes-Lima et al.,2015; Lighton and Schilman,2007).

Anoxic treatment exposure for 4-5 hours was able to cause 50% mortality of the most exposed stages of *T.urticae* and 4th stage of *S. littoralis* as shown in this paper. Thus, it could be confirmed by what occurred in case of anoxic effect on *Locusta migratoria* (Ravn et al.,2019), while exposure periods should be longer than 14 d to cause mortality of the insects of the stored products when CO2 concentration is below 40% (Sadeghi et al., 2011). Anoxia was able to kill locusts after exposure to for 4h at 30°C 4h of anoxia by 5-fold elevation of hemolymph [K^+] and 2-fold reduction of $Na^{+.}$ Additionally, marked cellular damage and muscle damage were associated with halved content of hemolymph water, reduction of both muscle ATP of normoxic values to ≤3%, and hemolymph pH to 0.8 units (Ravn et al.,2019).

On the other hand, insects of the stored products as maize weevils (*Sitophilus zeamais* Linnaeus), in hermetic conditions under the steady decrease of O_2 to 0% in 6–9 days, produced the number of offspring lower than weevils in non-hermetic conditions (Moreno-Martinez et al., 2000).

Also, anoxic LT50s showed that eggs tolerated such conditions better than adult females of *T.urticae* (Suzuki et al., 2015) and that was in the same trend with the present study which arranged tolerance of anoxia upon LT50s as the following: Eggs > Adult males > Adult Females. Additionally, findings through this paper have confirmed the notion that anoxia with high-CO2 stress motivates the creation of NADPH and accordingly, glutathione, which are engaged with the defense against the toxic impacts of reactive oxygen species (Boardman et al., 2011).
It could be understood clearly when it is known that NADPH involves in the synthesis of nucleotide, cholesterol and fatty acids (Feron, 2009). Moreover, it was detected that trehalose which is the essential carbohydrate in insects and plays a significant role in the development of the insect and all physiological requirements by being an essential vitality source just as by moderating abiotic stressors (Shukla et al., 2015), but it also would be a new entrance for a new pesticide with an innovated mode of action. Anoxia caused mortality by blocking the trehalose balance (Tang et al., 2018) through the inhibition of Trehalose-6-phosphate synthase (TPS) and Trehalose-6-phophate phosphatase (TPP) in affected pests (Kern et al., 2012).

Likewise, exposed non-diapausing females of *Tetranychus sp.* were able to control their peritremes by modulation the position of the stylophore, which means their ability to reduce the pressure of required O2 under the reduction of lethal effect in the tracheal system (McEnroe,1961).

CONCLUSION

Purposefully, anoxia could be a successful new treatment to can control specific pests by many mechanisms to cause mortality as a result of their exposure in IPM programs. Thereafter, anoxic stress is affected certain stages of exposed pests' development. Resulted alterations were linked directly with exaggerated reactive oxygen species because of increased exposure to anoxia.

REFERENCES

Armstrong, G. A. B., Xiao, C., Krill, J. L., Seroude, L., Dawson-Scully, K. and Robertson, R. M. (2011) Glial Hsp70 protects K^+ homeostasis in the Drosophila brain during repetitive anoxic depolarization. PLoS ONE, 6: e28994. DOI:10.1371/journal.pone.0028994

Bayley, J. S., Winther, C. B., Andersen, M. K., Grønkjær, C., Nielsen, O. B., Pedersen, T. H. and Overgaard, J. (2018) Cold exposure causes cell death by depolarization-mediated Ca^{2+} overload in a chill-susceptible insect. Proc. Natl. Acad. Sci. USA 115, E9737-E9744. DOI:10.1073/pnas.1813532115

Bergamini, C., Gambetti, S., Dondi, A. and Cervellati, C. (2004) Oxygen, reactive oxygen species, and tissue damage. Curr. Pharm. Des., 10: 1611-1626. DOI:10.2174/1381612043384664

Boardman, L., Sørensen, J. G., Johnson, S. A., and Terblanche, J. S. (2011) Interactions between controlled atmospheres and low-temperature tolerance: a review of biochemical mechanisms. Front. Physiol., 2:92. DOI: 10.3389/ fphys.2011.00092

Callier, V., Hand, S. C.,Campbell, J. B., Biddulph, T. and Harrison, J. F.(2015) Developmental changes in hypoxic exposure and responses to anoxia in Drosophila melanogaster. J. Exp. Biol., 218: 2927-2934. DOI:10.1242/jeb.125849

Campbell, J. B., Andersen, M. K., Overgaard, J. and Harrison, J. F. (2018) Paralytic hypo-energetic state facilitates anoxia tolerance despite ionic imbalance in adult Drosophila melanogaster. J. Exp. Biol., 221: jeb177147. DOI:10.1242/jeb.177147

Dittrich, V. (1962) A comparative study of toxicological test methods on a population of the two-spotted spider mite (*T.urticae*). J. Econ. Entomol., 55 (5): 644- 648.

Feron, O. (2009) Pyruvate into lactate and back: from the Warburg effect to symbiotic energy fuel exchange in cancer cells. *Radiother.* Oncol, 92:329–333. DOI: 10.1016/j.radonc.2009.06.025

Golestan, M.N. and Rahimi, H. (2017) Effect of modified atmosphere on obvious and hidden contamination to control of *Plodia interpunctella* (Hubner) and *Tribolium confusum* Jacquelin Du Val inside highly permeable packages. J. Biopesticides, 10(2): 83-89.

Harrison, J. F., Greenlee, K. J. and Verberk, W. C. E. P. (2018) Functional hypoxia in insects: definition, assessment, and consequences for physiology, ecology, and evolution. Annu. Rev. Entomol., 63: 303-325. DOI:10.1146/annurev-ento-020117-043145

Hermes-Lima, M.,Moreira, D. C., Rivera-Ingraham, G. A., Giraud-Billoud, M., Genaro-Mattos, T. C. and Campos, É. G.(2015) Preparation for oxidative stress under hypoxia and metabolic depression: revisiting the proposal two decades later. Free Radic. Biol. Med., 89: 1122-1143. DOI:10.1016/j.freeradbiomed.2015.07.156

Hochachka, P. W. (1986) Metabolic arrest. Intensive Care Med. 12, 127-133. DOI:10.1007/BF00 254926.

Kern, C., Wolf, C., Bender, F., Berger, M., Noack, S., Schmalz, S., et al. (2012) Trehalose-6-phosphate synthase from the cat flea *Cetenocephalides felis* and *Drosophila melanogaster*: gene identification, cloning, heterologous functional expression and identification of inhibitors by high throughput screening. Insect Mol. Biol., 21: 456–471. DOI: 10.1111/j.1365-2583.2012.01151.x

Lighton, J. R. B. and Schilman, P. E. (2007). Oxygen reperfusion damage in an insect. PLoS ONE 2, e1267. DOI:10.1371/journal.pone.0001267

McEnroe, W.D. (1961) The control of water loss by the two-spotted spider mite (*Tetranychus telarius*). Ann. Entomol. Soc. Am., 54:883–887.

Mitcham, E., Martin, T., and Zhou, S. (2006) The mode of action of insecticidal controlled atmospheres. Bull. Entomol. Res., 96:213–222.

Montzka, S., and Reimann, S. (2011) Chapter 1: ozone-depleting substances (ODSs) and related chemicals. In: World Meteorological Organization (ed) Scientific assessment of ozone depletion: 2010, Global Ozone Research and Monitoring Project—Report No. 52. Geneva, pp 1.1–1.108.

Moreira, D. C., Venancio, L. P. R., Sabinoa, M. A. C. T. and Hermes-Lima, M. (2016). How widespread is preparation for oxidative stress in the animal kingdom? Comp. Biochem. Physiol., 200:64-78. DOI:10.1016/j.cbpa.2016.01.023

Moreno-Martinez, E., Jiménez, S., and Vázquez, M. E. (2000). Effect of *Sitophilus zeamais* and *Aspergillus chevalieri* on the oxygen level in maize stored hermetically. J. Stored Prod. Res., 36: 25–36. DOI: 10.1016/S0022-474X(99)00023-5

Nakano, Y. and Asada, K. (1981) Hydrogen Peroxide Is Scavenged by Ascorbate-Specific Peroxidase in Spinach Chloroplasts. Plant and Cell Physiology, 22: 867-880. DOI: 10.1093/oxfordjournals.pcp.a076232

Nicolas, G., and Sillans, D. (1989) Immediate and latent effects of carbon dioxide on insects. Ann Rev Entomol.34:97–116. https://www.annualreviews.org/doi/pdf/10.1146/annurev.en.34.010189.000525

Ravn, M. V., Campbell, J. B., Gerber, L., Harrison, J. F., and Overgaard, J. (2019). Effects of anoxia on ATP, water, ion and pH balance in an insect (*Locusta migratoria*). J. Experimental Biol.,222: jeb190850. DOI: 10.1242/jeb.190850

Ristaino, J.B., and Thomas, W. (1997) Agriculture, methyl bromide, and the ozone hole: can we fill the gaps?. Plant Dis., 81:964–977.DOI: 10.1094/PDIS.1997.81.9.964

Rodgers, C. I., Armstrong, G. A. B., and Robertson, R. M. (2010). Coma in response to environmental stress in the locust: a model for cortical spreading depression. J. Insect Physiol., 56: 980-990. DOI: 10.1016/j.jinsphys.2010.03.030

Rodriguez, E. C., and Robertson, R. M. (2012). Protective effect of hypothermia on brain potassium homeostasis during repetitive anoxia in *Drosophila melanogaster*. J. Exp. Biol., 215: 4157-4165. DOI: 10.1242/jeb.074468

Sadeghi, G. R., Pourmirza, A. A., and Safaralizade, M. H. (2011). Effects of nitrogen and phosphine mixtures on stored-product insects' mortality. Afr. J. Biotechnol., 10: 6133–6144. DOI: 10.5897/AJB11.080

Seki, M., and Murai, T. (2012a) Responses of five adult thrips species (Thysanoptera; Thripidae) to high-carbon dioxide atmospheres at different temperatures. Appl. Entomol. Zool., 47:125–128. DOI: 10.1007/s13355-012-0098-6

Seki, M., and Murai, T. (2012b) Insecticidal effect of high carbon dioxide atmospheres on thrips eggs oviposited in plant tissue. Appl. Entomol. Zool., 47:433–436. DOI: 10.1007/s13355-012-0138-2

Shukla, E., Thorat, L. J., Nath, B. B., and Gaikwad, S. M. (2015). Insect trehalase: physiological significance and potential applications. Glycobiology, 25: 357–367. DOI: 10.1093/glycob/cwu125

Storey, K. B., and Storey, J. M. (1990). Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. Q. Rev. Biol., 65: 145-174. DOI:10.1086/416717

Suzuki, T., Wang, C.H., Gotoh, T., Amano.H., and Ohyama, K. (2015). Deoxidant-induced anoxia as a physical measure for controlling spider mites (Acari: Tetranychidae). Exp. Appl. Acarol., 65:293–305. DOI 10.1007/s10493-015-9881-8

Tang, B., Wang, S., Wang, S.G., Wang, H.J., Zhang, J.Y. and Cui, S.Y. (2018) Invertebrate Trehalose-6-Phosphate Synthase Gene: Genetic Architecture, Biochemistry, Physiological Function, and Potential Applications. Front. Physiol., 9:30. DOI: 10.3389/fphys.2018.00030

Van Lenteren, J.C. (2000) A greenhouse without pesticides: fact or fantasy?. Crop Prot., 19:375–384. DOI: 10.1016/S0261-2194(00)00038-7

Cited as:

ABD EL-WAHAB, R. (2020). Anoxia as a treatment against *Tetranychus urticae* and *Spodoptera littoralis*. *International Journal of Computational and Biological Sciences, 1*(1):20-29.
https://www.ijcbs.org/index.php/ijcbs/article/view/3