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THE INFLUENCE OF CERTAIN PHYSICAL STRESSES ON PULAL MORTALITY OF *CULEX PIPIENS* COMPLEX L.
II. EFFECT OF EXPOSURE TO OXYGEN AND PARTIAL VACUUM.

By

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تأثير بعض الضغوط الطبيعية على أحداث الوفاه في بعوضة الكيولكس بيبنز
٢ - تأثير الاكسجين والتفريغ الجزئي

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يعتبر هذا البحث أول محاولة في سلسلة البحوث التي يدرس فيها تأثير كل من الاكسجين والتفريغ الجزئي ودرجة الحرارة والرطوبة النسبية كل على حدة أو مع أشعة جاما على بعوضة الكيولكس بيبنز .

ودلت نتائج البحث أنه عندما عرضت العذارى للأكسجين لفترات مختلفة تتراوح بين ١/٢ إلى ٤٨ ساعة ازدادت نسبة الوفيات مع زيادة مدة التعريض حتى وصلت إلى حوالي ٥٠٪ بعد ٢٤ ساعة وإلى أكثر من ٦٠٪ بعد ٤٨ ساعة .

وعند تعريض العذارى للتفريغ الجزئي بدرجات من ٠,٠١ إلى ٠,١ (تورشيللي) ولفترات من ١/٢ إلى ٤٨ ساعة زادت نسبة الوفيات بزيادة درجة التفريغ وأيضاً بزيادة فترة التعريض .

Key Words: Effect of oxygen, partial vacuum, *Culex pipiens.*

ABSTRACT

The results indicated that when pupae were exposed to oxygen atmosphere for different periods ranging from 1/2 - 48 hours, the pupal mortality increased with increasing the exposure periods. The mortality reached to about 50% after 24 hours and more than 60% after 48 hours.

When the pupae were exposed to partial vacuum (0.01-0.1 torr) for different exposure times (1/2 - 48 hours) the percentage mortality increased gradually with increasing the degree of evacuation and with increasing the exposure periods.

INTRODUCTION

The present paper is the first trial in a series of papers that deals with the influence of certain physical stresses i.e. oxygen, partial vacuum, temperature, relative humidity and gamma radiation applied separately or combined, on some biological aspects of the mosquito, *Culex pipiens* complex L.

In the present work, oxygen and partial vacuum are studied as to their effects, when applied separately on the mosquito pupal mortality.

MATERIALS AND METHODS

The initial populations of *Culex pipiens* complex L. used in the present experiments were obtained from laboratory stock cultures reared in the Middle Eastern Radioisotope Centre, Dokki, under a controlled conditions of 24-28°C and 65±5% relative humidity. The rearing technique described by Abdel-Malek and Ahmed (1979) was followed.

Oxygenation Treatments:

For oxygenation treatments, samples of newly formed pupae were placed over water-soaked cotton pads, into the bottom of a glass test tube (5 x 20 cm) in which air was replaced by a continuous flow of oxygen (O_2) received from large steel bottles (50 liters), under high pressure of 1.5 atmosphere. After the tube had been filled completely with oxygen, it was closed tightly so that no air could get in.

The test tube was sealed at the bottom, the top end was covered with a metallic cover having two openings (the inlet and outlet) ending in two rubber connections with valves, one to control the current of oxygen flow into the tube and the other is free for air to come out of the tube. The oxygen pressure inside the tube was equal to the normal air pressure.

The exposure of pupae to oxygen was carried out for different times, after which the pupal mortality was recorded. The optimum time of exposure to the gas was determined according to pupal mortality after 48 hours.

Partial Vacuum Technique:

To elucidate the effect of partial vacuum, the following treatment technique was followed. A sample of newly formed pupae was placed over water-soaked cotton pad into the bottom of the test tube which was previously applied for oxygenation in this study. The inlet tube was connected to high vacuum pump with ultimate vacuum 0.00001 torr, free air displacement 3 m3/h. The outlet tube was connected to Edward gauge for adjusting and accurately measuring the vacuum.

The operation involved was as follows: after transferring the sample of pupae. in the test tube, the metal cover was tightly closed. Then, the pump was operated whereas the valve of the inlet tube was simultaneously opened and the valve of the outlet tube was closed. Over periods of 5, 10 and 15 mins., the valve of the inlet tube was closed and the pump stopped, then the valve of the outlet tube was opened to permit the gauge to measure the vacuum. That field produced varying currents of air which were determined at 0.01, 0.05 and 0.10 torr at 5, 10 and 15 minutes, respectively. The partial vacuum intensity was checked before and after treatment. The optimum partial vacuum was determined by knowing the median lethal does in the life time.

Multiple range statistical analysis were made according to Witte (1989) and Renner (1970).

RESULTS AND DISCUSSION

For the determination of median lethal dose after 24 and 48 hours for each treating media (i.e., oxygen, partial vacuum) applied to *Culex pipiens* L. pupae, a lot of 200 newly formed pupae were exposed to each medium for different time periods i.e., O (control), 0.5, 1, 2, 4, 8, 16, 24, 32 and 48 hours. Immediately at the end of these periods pupal mortality was recorded then were immersed in water under ordinary room conditions (24-28°C and 65±5% R.H.). The pupal mortality counts were also taken after 24 hours (24 h. LT values), and 48 hours (48 h. LT values). Three replicates were performed for each test.

Figures (1, 2) indicate the relationship between the exposure period and pupal mortalities immediately, 24 hours and 48 hours after exposure to oxygen and partial vacuum, respectively.

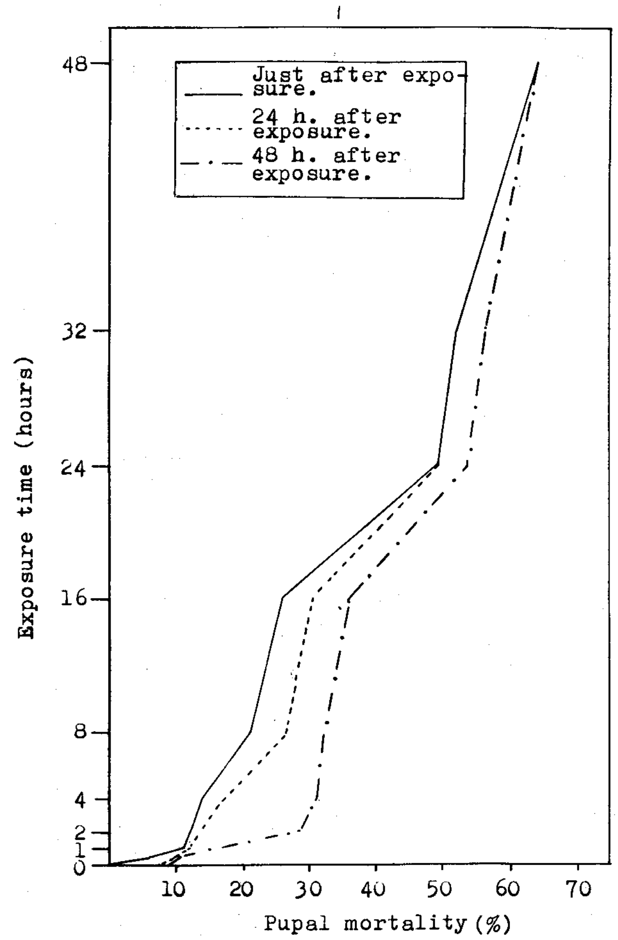

Fig. 1: Effects of oxygen alone on percentage mortality of *Culex pipiens* L. newly formed pupae.

Effect of Oxygen:

From the data presented in Fig. 1., it can be observed that mortality measurements immediately after exposure (Case 1), were significantly increased with increasing the exposure period to oxygen, showing the time-response relationship and indicating the active fatal nature of oxygen on *Culex pipiens* complex L. pupae. The percentage mortality reached its maximum after treatment for 48 hours (64.00%), while it was insignificantly increased to 5.83% after the first half hour of exposure, compared to 0.00% in the controls.

The pupal mortality counts recorded after 24 hours (24 h. LT values - Case 2) illustrate that, the median lethal time after 24 hours (24 h. LT_{50}) was well demonstrated after 24 hrs. of exposure to the oxygen, at which the percent mortality of pupae reached approximately its median level (49.83%) versus 6.66% for the corresponding controls. These levels of mortalities (Case 2) showed highly significant ($P<0.01$) differences if compared with 48 h. LT values (Case 3). However, there were insignificant differences between magnitudes recorded after treatment immediately (Case 1) and 24 h. LT values (Case 2).

The data concerning the 48 h. LT values assessed by the measurements of pupal mortality percentages after a period of 48 hours (Case 3), showed that, the response of the insect to the oxygen gas was considerably increased with increasing the time of exposure where highly significant differences ($P<0.01$) were recorded.

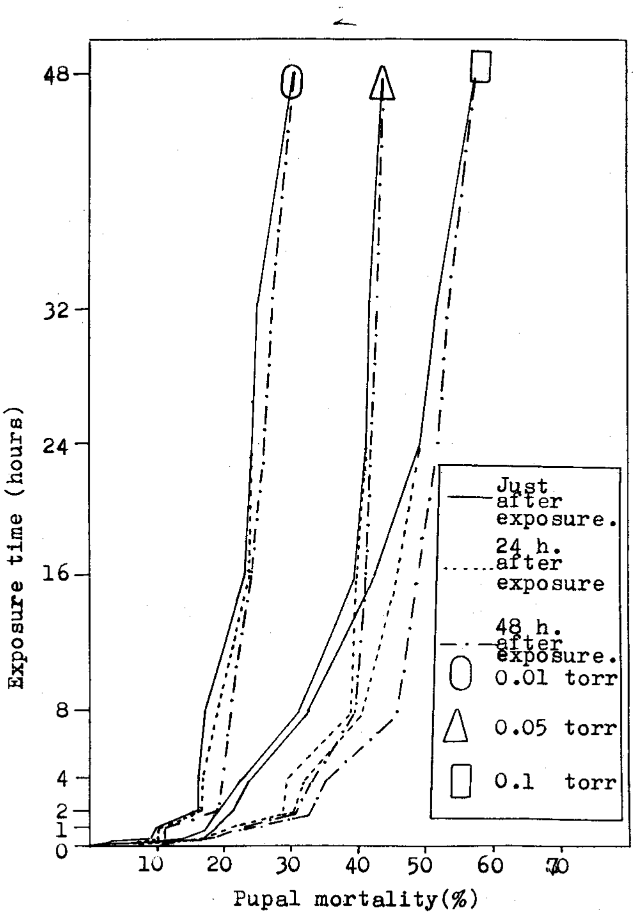

Fig. 2: Effects of partial vacuum alone on percentage mortality of *Culex pipiens* L. newly formed pupae.

Effect of Partial Vacuum:

Fig. (2) indicates the mortality percent of the treated pupae with partial vacuum at different intensities (0.01, 0.05 and 0.10 torr). The data illustrate clearly that, there was an increase in the effect of partial vacuum on the mortality of pupae of *Culex pipiens* L. with increasing its intensity at all exposure times concerned. However, there were slight variations and insignificant differences in the initial kill at 0.01 torr after exposure for short periods (0.5 or 1 hour) at all times after exposure, then the increase in the pupal mortality was rapid and significantly varied ($P<0.01$) as the exposure periods was prolonged. However, no LT_{50} value could be established since the percentage of mortality did not indicate more than 24.33% compared to 7.00% in the corresponding controls. On the other hand, at 0.01 torr no significant differences in mortality values were detected between pupae at all cases (times after beginning of exposure). Moreover, no detectable significant differences between mortality values within high exposure periods (16, 24, 32 and 48 hours) were observed.

As to the effects of partial vacuum with 0.05 torr, data reported in Fig. (2) show that, treatment of pupae for even short periods resulted in a significant increase in pupal mortality than in the corresponding control. The mortality increased with increasing the exposure period but it did not reach the 50% neither after 24 hours (Case 2) nor 48 hours (Case 3) after beginning of exposure (41.66 and 44.00% after 24 and 48 hours, respectively). The differences in the mortality values within high levels of exposure (16, 24 or 48 hours) were statistically insignificant compared to percentage mortalities recorded immediately after exposure and significant compared to that recorded after 48 hours from treatments.

Results of the mortality percent after treatment by using 0.1 torr, showed that, pupal mortality increased as the time of exposure increased at all cases of observations. After exposure for 24 hours, the percentage mortality reached the median level (49.50%) after 24 hours of exposure.

Generally, the differences within percentages mortality at all exposure periods within the three cases of observations were statistically significant (at the probability level $P = 0.001$).

From the above results it was shown that oxygen was fatal to the mosquito pupae and the percent pupal mortality was increased with increasing the time if exposure. The obtained results are to a high extent similar to those obtained by many workers, who stated, oxygen at high tensions is toxic to insects, as it is to many other animals which have been examined. For instance, oxygen reduced viability of adults *Drosophila azteca* (Williams and Beecher, 1944) and delayed-embryonic development of *Drosophila melanogaster* (Glass and Plaine, 1952). In *Habrobracon*, only the prepupae and the white pupae were sensitive to 1 atm. of oxygen; earlier and later stages were unaffected. The effects seen were prevention of emergence in some cases and wing and antennal abnormalities in others (Clark and Herr, 1954). In *Anagasta*, the Mediterranean flour moth, 1 atm. of oxygen allowed pupal development, but the adults could not emerge (Clark and Cristofalo, 1961). Sensitivity of *Tribolium confusum* males and females to pure O_2 at 1 atmosphere was measured by Lee and Ducoff (1983). They found that, sensitivity to oxygen increased markedly with age, the reciprocal to the LD_{50} exposure time was a linear function of age.

It was found that, there was an increase in pupal mortality with increasing the degree of vacuum or with increasing the time of exposure to the vacuum. The results obtained by other authors on other insects were generally similar to our results on *Culex pipiens*, for example, the metabolic effect of hypoxia and anoxia on the larvae of *Chironomus thummi* and *Culex pipiens* was investigated by Redecker and Zebe (1988), who found that, in *C. thummi*, anoxia resulted in a characteristic decrease of ATP and P-arginine concentrations and in an accumulation of alanine and lactate within 60 minutes. These changes continued during prolonged incubation but at lower rates. Ethanol, the major product during long term anoxia, was largely excreted into the ambient water. A significant accumulation of these metabolites occurred only at a PO_2 of 7 torr. *Culex* larvae were shown to have a very low anaerobic capacity and a high rate of lactate accumulation.

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