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THE RELATIVE TOXICITY OF HYDROGEN CYANIDE, CHLORPICRIN AND ETHYLENE OXIDE TO THE EGGS, NYMPHS AND ADULTS OF THE COMMON BEDBUG (*Cimex Lectularius*).

by

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INTRODUCTION

Hydrogen cyanide has been used as a successful fumigant against bedbugs for the past twenty-five years, but very little work has been done to determine accurately the actual concentration of gas necessary to insure good results. It is known that a great deal of gas is lost through leakage, absorption, and other ways, but as far as can be determined, no one has found the lethal concentration of gas necessary for *Cimex lectularius*, nor has the median lethal concentration been determined. Ethylene oxide and chlorpicrin have come into general use as fumigants only recently, but their growing popularity makes studies into their efficiency desirable.

ACKNOWLEDGMENT

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REVIEW OF LITERATURE

The first use of hydrogen cyanide as a fumigant against household insects was recorded by Howard (7), who stated that Marlatt used hydrogen cyanide against book lice in 1898. Herrick (6) used hydrogen cyanide for fumigating dormitories and obtained excellent results.

Coleman (4) was one of the first to determine the relationship between the concentration of fumigant, time of exposure and insect mortality. He investigated the toxic properties of hydrogen cyanide against scale insects. Hydrogen cyanide was generated and passed through a series of purifying tubes into a Novy gas jar, in which his experimental insects were placed. From this fumigation chamber the gas was withdrawn into a solution of potassium hydroxide, and titrated with silver nitrate to determine the amount of hydrogen cyanide present. His measurement of toxicity was based on 100% mortality obtained over periods of from 15 to 60 minutes. He found that a great deal of hydrogen cyanide used in actual fumigation was lost through leakage and thus had no action upon the insects. His results are questionable for two reasons. First, a 100% mortality is theoretically unobtainable, and under experimental conditions, the exact concentration of gas causing 100% mortality would be hard to determine. Also the concentration of gas causing 100% mortality in 15 minutes would be much greater than that causing the same mortality in 60 minutes.

Niefert and Garrison (11) determined the concentrations of a number of toxic agents, which caused 100% mortality in 24 hours, using hydrogen cyanide as a standard for comparing the toxicity of other gases. They used meters to measure and regulate the flow of mixed gas and air into a 5-liter jar, which served as a fumigation chamber. Lethal concentrations were expressed as percentages. Here again the same objections prevail as in Coleman's work.

Strand (14) compared different methods of determining relative toxicity. He determined the 5-hour median lethal concentrations of hydrogen cyanide, chlorpicrin and ethylene oxide for the Confused flour beetle (Tribolium confusum Duv.). He found that if the time of exposure and the temperature during exposure were kept constant, and only the concentration of the fumigant varied, he had an ideal method for comparing the relative toxicity of various fumigants. By following Strand's method, one may not only compare the effect of different agents on one species of insect, but also have a basis for the comparison of a fumigant on different insects. His experimental method differed but slightly from that used in the experiments described herein. He determined the concentration of hydrogen cyanide present in the fumigation chamber by absorbing the gas in potassium hydroxide and titrating with silver nitrate.

Page (12) and Lubatti (9) evolved a method for measuring the concentration of hydrogen cyanide and ethylene oxide present at any time during actual fumigation. They used an evacuated flask fitted with a capillary tube which could be broken off at any time by closing an electric circuit. The absorbent used for hydrogen cyanide was 50 cc. of 0.1 N sodium hydroxide. At the end of the experiment the sodium hydroxide was titrated with silver nitrate to determine the amount of hydrogen cyanide absorbed. The absorbent for ethylene oxide was magnesium chloride. The contents of the flask were titrated at the end of the experiment with dilute sodium hydroxide, using methyl orange as an indicator.

Moore (10), who was one of the first to use chlorpicrin, determined the minimum lethal dose of chlorpicrin necessary to kill 100% of the insects. He used the bean weevil (Bruchus obtectus S.), the Angumois grain moth (Sitotroga cerealella Oliv.), the Indian Meal moth (Plodia interpunctella

Hbn.), Mediterranean flour moth (Ephestia kuehniella Zell.), and the Confused flour beetle (Tribolium confusum Duv.).

Bertrand, Brocq-Rousseau and Dassonville (1) determined the toxicity of chlorpicrin against bedbugs (C. lectularius) and weevils.

Lindgren and Shepard (8) modified Strand's experimental method. They found that changes in humidity caused the median lethal concentration of chlorpicrin to vary rather significantly for the eggs of the Confused flour beetle (T. confusum Duv.), but had no effect on the adults. They also found that changes in humidity had no effect on the median lethal concentration of ethylene oxide for eggs and adults of the beetle. Brown (3) used a mixture of carbon dioxide and ethylene oxide to fumigate cockroaches (Blatella sp.) and bedbugs (C. lectularius). Hase (5) conducted experiments with ethylene oxide as a fumigant for the bedbug (C. lectularius).

EXPERIMENTAL METHOD

The apparatus used in these experiments is the Strand apparatus as modified by Lindgren and Shepard (8). Erlenmeyer flasks of approximately 6.4 liters capacity were used as fumigation chambers. Each flask was fitted with a ground glass stopper, through which were sealed two glass tubes, each supplied with a stopcock.

The hydrogen cyanide used in these experiments was obtained through the reaction of sodium cyanide with sulphuric acid in the presence of water. The gas was cooled in a condensing tube and collected in a flask buried in ice. The resulting liquid was redistilled several times until the freezing point of pure hydrogen cyanide was reached.

The HCN was measured into the fumigating flasks as a gas. A gas burette calibrated in 0.01 cc. was filled with mercury and a leveling bulb attached. The tube containing the liquid cyanide was attached to the burette and warmed gently, the leveling bulb lowered and the gas drawn into the burette. The pressure was equalized, the volume of gas measured, temperature and pressure recorded, and then the gas was drawn into the partially evacuated flask. The weight of gas in a given volume was determined by means of the formula: $PV = \frac{g}{M} RT$, where P is the atmospheric pressure, V is the volume of gas, g is weight of gas, M is the molecular weight of the gas, R is the gas constant; 82.06 c.c. atm. per mol deg.; and T is the temperature at which the volume of gas is measured. This formula is derived from the fundamental gas laws and, in general, is accurate to within 5%. In order to check the results obtained with this formula, a given volume of gas was absorbed in a solution of potassium hydroxide, and titrated with silver nitrate. The results checked within 1%.

Chlorpicrin was measured as a liquid with a micropipette calibrated in 0.001 cc.

Ethylene oxide was measured as a gas in the same way as was hydrogen cyanide. It was assumed that the error in measuring would not exceed 1%.

The eggs, nymphs and adults of the common bedbug (C. lectularius), were used in these experiments. The eggs used were two days old; nymphs were of second and third instars. All stages were kept at 27°C. For each experiment between 25 and 30 individuals of each stage were used, since Trevan (15) showed that the use of less than 30 test individuals causes a considerable error, but the use of more than this number contributes little to the accuracy of the experiment. Bliss (2) showed by statistical methods that as low as 10 individuals could be used with no loss of accuracy between percentage mortalities of from 38 to 61%.

The test insects were placed in bolting cloth cages, put into the fumigation chambers, which were then partially evacuated, and the fumigant introduced. The flasks were then placed at 25°C. and left for 5 hours. At the end of that time the insects were removed from the cages and placed in glass vials at 27°C. Mortality counts were made in 24 hours and again in 48 hours. In all cases it was found that the mortality did not change after 24 hours. No correction was necessary since none of the controls died. The fumigated eggs were placed at 27°C. with checks, and inspected every day. Normal bedbug eggs kept at 27°C. hatch in 5 days, so no counts were made after the expiration of that time. The percentage mortality was calculated by means of Abbott's formula: $\frac{x - y}{x}(100)$; where x is the percent hatch in the check and y the percent hatch in the treated lot.

DISCUSSION OF RESULTS

From these results it will be seen that the three fumigants used here differ greatly in their action upon the different stages of the common bedbug.

TABLE I. Five-hour Median Lethal Concentrations for Eggs, Nymphs and Adults of *Cimex lectularius*.

Fumigant	Eggs-Mg./liter	Nymphs-Mg./liter	Adults-Mg./liter
Hydrogen cyanide	0.1075 mg.	0.319 mg.	0.3325 mg.
Chlorpicrin		2.180 mg.	0.582 mg.
Ethylene oxide	0.217 mg.	1.31 mg.	1.88 mg.

From an inspection of fig. 2 it may be seen that no median lethal concentration of chlorpicrin could be determined for the eggs of *C. lectularius*, although it seems to lie between 5.0 and 6.0 mg. per liter. The nymphs and adults appear to be two or three times as susceptible to chlorpicrin as the eggs.

Hydrogen cyanide and ethylene oxide appear to have nearly the same toxicity to the eggs of the bedbug, but almost 6 times as much ethylene oxide is needed to produce 50% mortality in nymphs and adults as compared to hydrogen cyanide.

CONCLUSIONS

From an inspection of the results obtained in these experiments, it is indicated that hydrogen cyanide is a better fumigant for all stages of *C. lectularius* than either ethylene oxide or chlorpicrin. Ethylene oxide would be preferable to chlorpicrin because of its action on the eggs of the bedbug. In refutation of the idea that the eggs of an insect are the most resistant stage, it was found that they are the least resistant to some of the fumigants used here. These experiments tend to disprove the theory that a second fumigation of HCN is necessary to kill the eggs of the common bedbug. In fact, it is quite the reverse. A second fumigation might be necessary to kill the adults.

SUMMARY

Experiments are described and results recorded for the effect of

hydrogen cyanide, chlorpicrin and ethylene oxide upon the eggs, nymphs and adults of the common bedbug (C. lectularius). The 5-hour median lethal concentration of each fumigant was determined. It is shown that HCN has a greater toxic action against all stages of this insect than either ethylene oxide or chlorpicrin.

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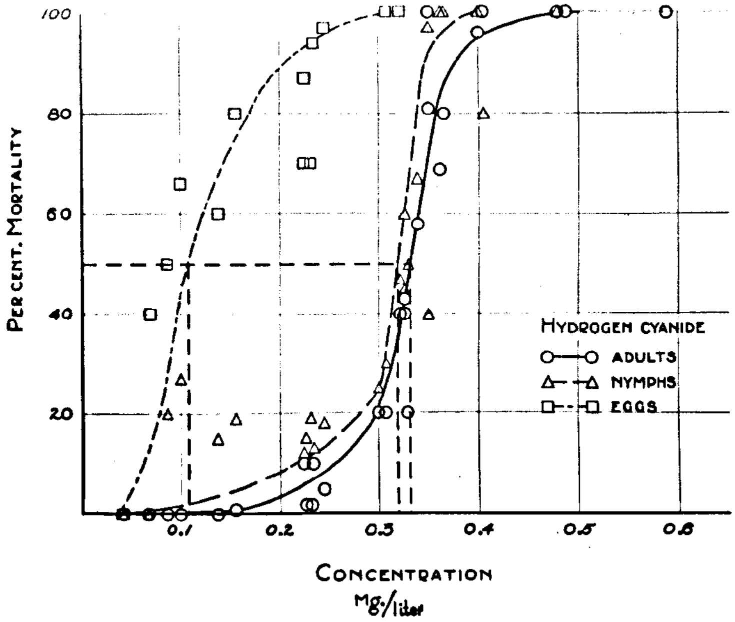

Fig. 1. Five-hour median lethal concentration of Hydrogen Cyanide.

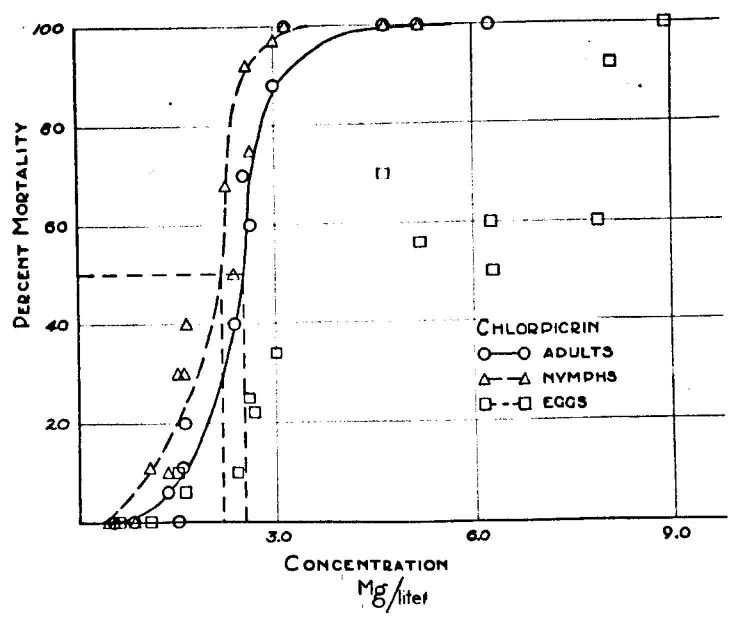

Fig. 2. Five-hour median lethal concentration of Chlorpicrin.

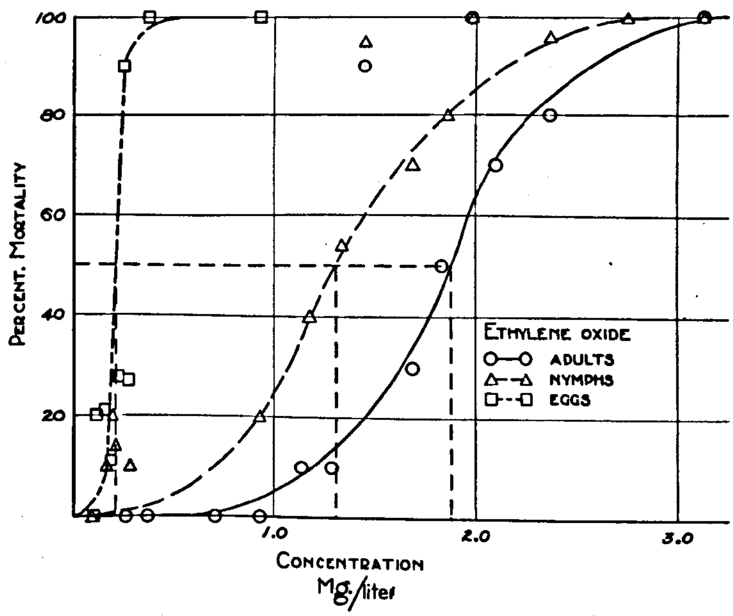

Fig. 3. Five-hour median lethal concentration of Ethylene Oxide.