Effect of a combination of a H_2O_2 fluid and permethrin by use of a fogger-technique against permethrin resistant and non-resistant bed bugs (Cimex lectularius)

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***Abstract*—A fogger was developed to fight against resistant bed bugs. A combination of permethrin and hydrogen peroxide delivered promising results in killing non-resistant and resistant bed bugs.**

Keywords— bed bugs, permethrin, hydrogen peroxide, resistance

I. INTRODUCTION

In developed nations, ectoparasites affecting humans such as e.g. lice are under constant treatment pressure and their occurrence is normally limited due to good countermeasures, strategies, and information of the people. Notwithstanding the common bed bug (*Cimex lectularius*) is exceptional among the ectoparasites and their populations has increased in the recent past almost everywhere [1,2]. Reasons for this, most probably include the restriction on chemicals (e.g. DDT) [1], globalization including travel habits of people as well as the transfer of goods [3] and last but not least selection for resistances especially against the pyrethroids [1,4]. Those changing conditions together with an effective biology composing of their ability to starve for more than 6 months, and their egg-laying rate of 5-7 eggs/week, their nidicolous lifestyle and their pheromonic based warning system [3,5] enabled the bed bugs their triumphal procession into public and private buildings, hotels, hostels etc. Mainly humans are affected, but also bats, birds, and pets can serve as hosts [3]. They role as vectors seems rather limited but recently the bed bugs were found to experimentally acquire *Trypanosoma cruzi*, a causative agent of Chagas-disease from mice and also infection of mice via bed bug faeces was possible [6]. Additionally, the bed bugs can experimentally be infected with 40 different human relevant pathogens [5,7] and also considering a potentially bridging of pathogens from the aforementioned non-human hosts to the humans. Nevertheless, even solely the nightly visits of those bugs may lead to dramatic physical and mental pressure for the persons concerned. Their occurrence can cause dermatological signs such as wheals, redness, and pruritus [8] and mental impacts like insomnia and anxiety state [3,5]. Fighting against this pest is restricted due to the limited chemicals compounds available on the market. Pyrethroids e.g. permethrin are presumable the most commonly used insecticides against bed bugs [3] leading to a wide distribution of selection for resistance. The pyrethroids affect the Na^+ channel and aim the neurological system of the insects. Besides this principal mode of action to delay Na^+ channel closure, it is known that pyrethroids elevate the cytotoxic effect due to increased oxidative stress of cells in the target species and free-radical-mediated lipid peroxidation [9]. The disinfection chemical hydrogen peroxide (H_2O_2) is very often used to disinfect surfaces against virus and bacteria operating with O_2 radicals. Hence, applying O_2 radicals on organisms and simultaneously increase the oxidative stress due to exposure with pyrethroids should both work together, aiming the same target. Consequently, a combined treatment of bed bugs with pyrethroids (e.g. permethrin) and H_2O_2 should increase the oxidative stress of the bed bugs cells, maybe leading to a synergetic effect in killing the bugs. As an application for the chemicals, a specialized fogger was used, which can distribute droplets of ~ 1 µm in size. This very small droplet size should help during the application in the field to reach the bed bugs in distant and hidden places and ensure a rapid uptake of the compounds via the stigmata.

II. MATERIALS AND METHODS

A. Fogger

For the application of the different compounds, a fogger was developed and provided by the company Braincon GmbH. This fogger was previously built to decontaminate surfaces in hospitals and clinics against viral and bacterial pathogens. In the previous setting, the H_2O_2 fluid was solely used and effective down to log 6 stage. One of the big advantages of this technology, unlike other foggers, this system is evaporating droplets smaller than 1 µm in diameter. This technology enables "dryer and cold" application, which is acting like gas and condensation is very much reduced. Furthermore, due to the small droplet size, the compounds are easier to be transported to very small cracks and crevices by diffusion.

B. *Originating and trial with bed bugs*

The bed bugs were obtained by the Cimex store (Cimex Store Ltd.) which can provide permethrin susceptible (London Lab strain) and permethrin resistant strains (Sweden Field strain). In preliminary studies on bed bugs with the H_2O_2 fluid alone, the poisoning was not promising (data not shown), so additional compounds were screened for combination with the prevailing system.

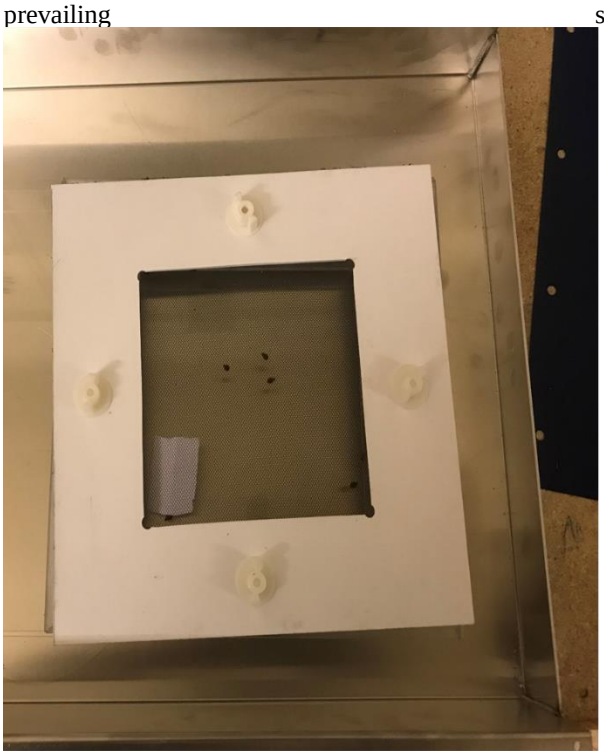

Fig. 1. Cage for keeping bed bugs in between the net and available for exposure.

C. *Composition of chemicals*

Although there are known resistant strains against the chemical compound permethrin (pyrethroid), this was chosen to continue. The reason, therefore, was the joint possession of an increase of oxidative stress while applying permethrin and H_2O_2 fluid and the potential of a synergistic effect as previously described.

D. *Exposure*

For the trial, the bed bugs were divided into groups of 15 individuals and fixed in between a net hold by a frame to hamper escaping of the bugs (Fig. 1). Those frames were then placed in a room of ~16 m^2 specially built for this testing purpose. In this room, the fogger was placed and the bed bugs were exposed for different intervals of 2 h and 4 h. For each time interval H_2O_2 fluid alone, permethrin alone and a combination of both (H_2O_2+ 3% permethrin) was tested.

E. *Bed bug viability*

To assess the effect of poisoning the bed bugs were checked for the viability after exposure to different intervals. They were classified into four groups depending on the symptoms they showed. Bed bugs, which were moving fast and coordinated, were classified as "no symptoms". Bed bugs showing uncoordinated walking and flipping to their back, but flipping back to the legs again were reported as "light symptoms". Bed bugs which were laying on the back, but moving their legs and trying to get up again without success, were grouped to "heavy symptoms" and bed bugs without any movement of legs were supposed to be "dead".

III. RESULTS

The bed bugs in London Lab strain (susceptible) showed heavy symptoms (dead or heavy) if treated with permethrin alone or in combination with the H_2O_2, whereas the H_2O_2 alone did not affect all the bed bugs in the 4 h group. Unfortunately, the 2 h group got lost due to technical reasons and therefore was excluded from the analysis (Fig. 2).

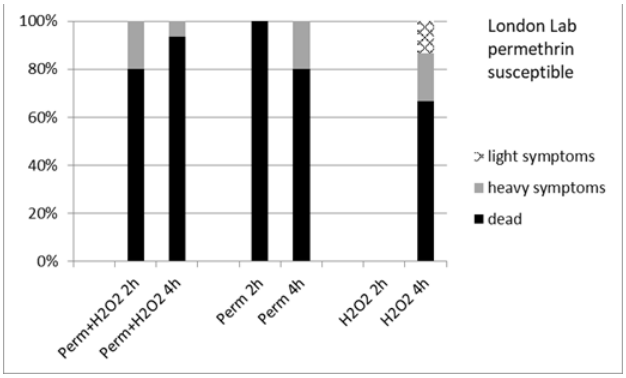

Fig. 2. Viability data of bed bugs from the London Lab strain (permethrin susceptible) after 2 h and 4 h for the combination of permethrin and H_2O_2 (Perm+H2O2), permethrin alone (Perm) and H_2O_2 alone (H2O2).

In the resistant Sweden Field strain, the H_2O_2 alone was not able to affect more than 60 % in the 2 h group and 50 % in the 4 h group. The remaining unaffected bugs did not display symptoms at all. The permethrin alone was effective in 60 % in the 2 h group and 50 % in the 4 h group, if including the light symptomatic bed bugs. The combination of permethrin and H_2O_2 affected all bed bugs in the 2 h group (light, heavy symptoms and dead) and in the 4 h (heavy symptoms, dead) (Fig. 3).

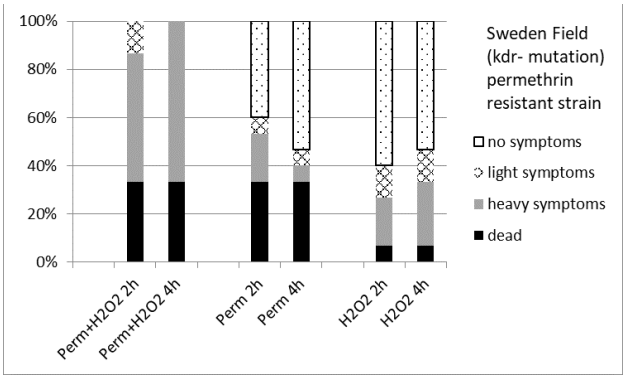

Fig. 3. Viability data of bed bugs from the Sweden Field strain (permethrin resistant) after 2h and 4 h for the combination of permethrin and H_2O_2 (Perm+ H2O2), permethrin alone (Perm) and H_2O_2 alone (H2O2).

IV. Discussion

In the susceptible strain (London Lab) there was no difference in the effect of permethrin alone or in combination with H_2O_2. The H_2O_2 alone had also an effect but some bugs additionally displayed light symptoms as well. In the resistant group (Sweden Field) the H_2O_2 was only effective on 40-50% of the treated individuals and in the permethrin-only group, 50-60 % reacted with symptoms, clearly confirming the occurrence of a mechanism of resistance. Even more in the longer exposed group (4 h), there were more bugs with no symptoms than in the 2 h group, which even may indicate a recovery from the poisoning. The inefficiency of permethrin can be easily explained by the known resistance (kdr mutation) against permethrin of this strain. The failure of the H_2O_2 could be partly explained by the lower effect of the H_2O_2 on the organism, as already seen with the London Lab strain. Additionally, this may be a hint, that the mechanism for the permethrin resistance in the Sweden Field strain also hampers the working mechanism of the H_2O_2.

The major mode in the permethrin resistance is a mutation on the pore protein [1], which enables resistant cells not to link with permethrin and keep the Na^+ channels closed, consequently being not affected by the permethrin. Additionally, the permethrin is increasing the oxidative stress [9], which is clearly in line with the working mechanism of H_2O_2, which overwhelms the cells with O_2 radicals. Why the application of the radicals is less effective in the permethrin resistant strain remains unclear. However, a combination of permethrin and H_2O_2 leads to a synergetic effect with heavy symptoms and dead bugs. Although compared to the London Lab strain, there were less dead bugs after 2 h and 4 h in the resistant groups. But after 12 h, all of the heavy symptomatic bugs were found dead (data not shown). This synergistic effect cannot be in association with the principal mode of the permethrin, which is the opening of Na^+ channel, because this is hampered or strongly reduced in the resistant strain (Fig. 4).

So, maybe the second mode of action of permethrin, the free-radical-mediated lipid peroxidation, might gain importance in permethrin resistant strains, especially in combination with a lot of provided radicals due to H_2O_2 (Fig. 4):

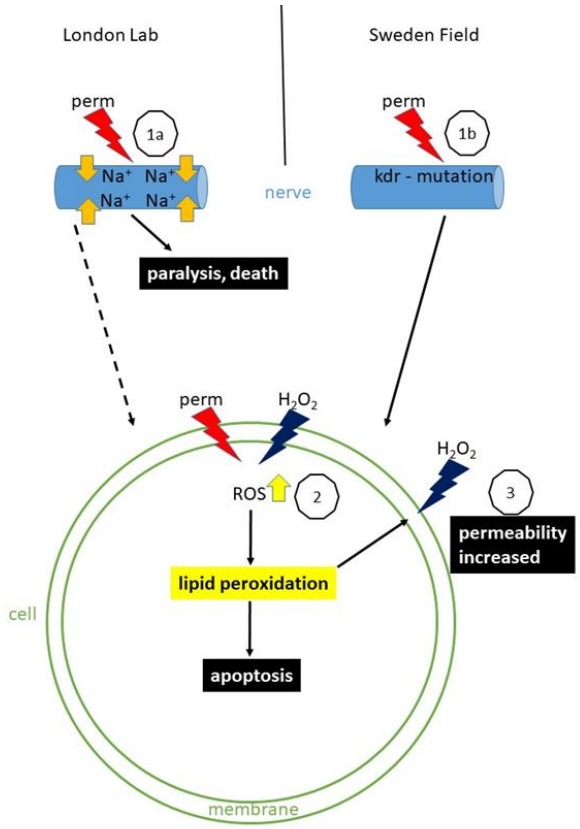

Fig. 4. Possible modes of intoxication: In the London Lab strain (permethrin susceptible) the major mode of action of the permethrin (perm) is the opening of the (Na^+) sodium channels (1a) which leads to paralysis and death. In the Sweden Field strain (permethrin resistant) the kdr-mutation hampers the opening of the sodium channels (1b), therefor lacking depolarization of the nerve cells. The second mode of action gains importance, which is the shift to a higher oxidative level in the cells (2) to more radicals (ROS = reactive oxygen species) due to applying permethrin and hydrogen peroxid (H_2O_2). Those radicals oxidize mainly lipids, but also proteins and lead to apoptosis. Lipid peroxidation in the membrane induces a higher permeability of the membrane, which in turn is also affecting the survival of the cell. (Modified after [10,11])

In theory, in the cells the balance of the free radicals is shifted to a higher level due to permethrin, therefor increasing the oxidative stress. Additionally, the application of O_2 radicals (in the H_2O_2 fluid) supports this shift further and kills the cells in the end by dysfunction due to accumulated oxidized proteins, lipids, DNA and thereafter apoptotic or necrotic mechanisms follow [10,11]. Additionally, it can be assumed that both, permethrin and H_2O_2, also influence the permeability of the cell membrane. Lipid peroxidation can occur in the membrane and react with proteins, consequently forming protein-lipid connections. Furthermore, oxidized proteins can lead to the disintegration of the cell membranes and increase their permeability [11].

V. Conclusion

The viability of the observed bed bugs suggests a synergetic effect if the H_2O_2 fluid is used together with the permethrin. This

becomes especially obvious regarding the bugs of the permethrin-resistant Sweden Field strain, which could also be killed with the combination. Further studies are needed to prove the effect on other resistant strains.

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