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A Review of the Repellency Properties of Pyrethrins

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I. Introduction

Pyrethrum is an insecticide created from the dried flower heads of plants in the genus *Chrysanthemum*. The six compounds responsible for the insecticidal activity are collectively known as pyrethrins, and are known individually as *Pyrethrin-I, Pyrethrin-II, Cinerin-I, Cinerin-II, Jasmolin-I,* and *Jasmolin-II.* Of these compounds, *Pyrethrin-I* is best known for mortality, and *Pyrethrin-II* is best known for knockdown activity (Sisay et al. 2001). These compounds are voltage gated sodium-ion channel disruptors acting on nerve cells. The inside of nerve cells is negative relative to the outside at rest. It is due to an imbalance between ions on either side of the cell membrane, creating a membrane potential, I.E. a high level of potassium and a low level of sodium inside the cell and more sodium than potassium outside the cell, and internally higher concentration of non-diffusible anions. When an action potential occurs after a stimulus, the membrane potential is temporarily reversed, allowing for sodium ions to enter the nerve cell through voltage-gated sodium channels and causing the nerve to fire. The level of sodium then begins to decrease, and potassium increases by active transport (K^+/Na^+ pump), returning to the ion concentrations of the cell's resting level after a brief hyperpolarization. Pyrethrins work by prolonging the open state of the voltage gated sodium channel, leading to paralysis (knockdown) and eventually mortality (Narahashi, T. 1971). If the concentration of pyrethrum is not high enough to cause knockdown, the insect can potentially leave the treated area (repellency) after experiencing sublethal effects. Pyrethrins are also known to be relatively harmless to mammals, especially when compared to other plant-based compounds (Maia and Moore 2011). It has an LD_{50} value of 1,200 to 1,500 mg/kg in rats. Nicotine by comparison has an LD_{50} value of 50 to 60 mg/kg in rats (Ware, G.W. 2000).

This review looks at the history and importance of pyrethrum as a repellent, test methods used to understand repellency, as well as the mechanisms responsible for causing an insect to be repelled. It focuses on urban and medical pests and the efficacy against them, as these pests tend to be disease vectors, or mechanical transmitters of disease, and have the highest need to be repelled.

II. History of Pyrethrum

Pyrethrum has a long history as a repellent and an insecticide. As more effective synthetic actives have been discovered and utilized, it has taken a smaller role.

According to Sun et al. (2020), the first record of pyrethrum use was 2000 years ago by the Chou Dynasty of China. Those *Chrysanthemum* flowers, from which pyrethrum is derived, then made their way to Europe along the Silk Road. Due to a lack of understanding around the specific species of flower during that time period, most agree that the official history of pyrethrum starts around 1847 with the description of *Chrysanthemum cinerariaefolium* when it was discovered in Croatia. One story shared by Casida (1973) is of a German woman in the Dalmatia region of Croatia, who was picking flowers as they bloomed, but would then throw them into a corner as they wilted. She then noticed several weeks later that the dried flowers were surrounded by several dead insects.

These flowers first made their appearance in the USA in 1885 when bales of dried flowers were shipped overseas. However, the first use of the flowers as a commercial product was thought to have occurred in the 1820s when a combination of *Chrysanthemum* species were most likely mixed to make a powder. It wasn't until around 1914 that the first major commercial planting of *Chrysanthemum* flowers occurred in Yugoslavia, with Japan becoming the main producer until 1939. During World War II, Kenya and other East African countries took over most of the production, where it continues to this day, along with Tasmania.

The first company to utilize pyrethrum in a form other than a power is thought to be the McLaughlin Gormley King Company, more familiarly known today as MGK. The company was an importer and miller of botanical materials and found that when the flowers were extracted with kerosene, the resulting liquid spray was much more effective than the powders. With this information, MGK hired Charles Gnadinger away from the Food and Drug Administration to study the potential specialization opportunities of pyrethrum. Upon reading the work of Staudinger and Ruzicka (1924), Gnadinger went on to identify the two primary active principles of pyrethrum and published a long series of papers on the subject, which laid the foundation for the present knowledge base of pyrethrum today (Casida, J.E. 1973).

III. Mosquitoes

Mosquitoes are often the primary target for repellents because they not only are a nuisance, but a serious disease vector in most parts of the world. For many people, repelling mosquitoes can be a life-or-death situation. The following articles look at the role pyrethrum plays in helping to protect against mosquito bites.

a. Study 1

In this study, Liu et al. (2021) looked at a combination of genetic, electrophysiological, and behavioral approaches at attempting to understand pyrethrum repellency in *Aedes aegypti*. Liu and team first looked at whether pyrethrum elicited spatial repellency by conducting an arm-in-cage test. The version used involved a special glove worn by the tester which had a mesh window consisting of two layers. When the second layer, closest to the skin, was treated with pyrethrum a significant reduction in mosquito lands was observed. Upon further investigation a dose-dependent response was observed. The team then used an electroantennogram (EAG), a device used to detect electric signals sent by the antennae, to find if being exposed to pyrethrum elicited a response. It was discovered that there was a response, indicating that pyrethrum repellency depends on the insect's olfaction system. A similar study was conducted on the mosquitoes' maxillary palps, another organ known to be part of the olfactory system, and no such response was present.

With this information in hand, a deep dive looking at specific olfactory receptor neurons (ORNs) followed. To do this, single sensillum recordings of antennal olfactory sensilla against various odorants were conducted. It was found that 9 sensilla had a measurable response to the panel of odorants, with two of them being the most responsive to pyrethrum specifically. It was then taken a step further and those two ORNs were then evaluated to see if individual odorant receptors (ORs) could be identified. By knocking out individual ORs it was discovered that AaOr31, when silenced, reduced pyrethrum repellency up to 60%.

Ultimately it was proven that repellency of mosquitoes by pyrethrum is through a dual-target mechanism. A minor component of pyrethrum, (E)-β-farnesene (EBF), activates AaOr31 causing repellency while at the same time the effects of pyrethrins activating the sodium channels of the nervous system also causes the insect to be repelled.

b. Study 2

Bandason (2018) conducted a similar study for her published dissertation which also showed repellency at the skin level against pyrethrum when tested in a hand-in-cage assay, and the elicit of a response in an EAG recording. Bandason then went on to test mosquitoes with known pyrethroid-resistance and found a decrease in repellency when compared to susceptible strains. This is significant because most work with resistant strains tends to focus on the ability to knockdown or kill the mosquitoes.

This study was conducted using both *Aedes aegypti and Anopheles gambiae*, two major disease vectors. The first test looked at the repellency effect against the mosquitoes in a hand-in-cage assay using various concentrations of pyrethrum. These were compared to DEET as a positive control. Both species reacted similarly and showed no difference in repellency between the treatment and control. Both species also showed an increase in repellency as the concentration of the treatment increased. To build on this test the author then conducted the study against a known pyrethroid resistant strain of *Ae. aegypti* known as the Puerto Rico (PR) strain. This study showed that while the mosquitoes continued to be repelled by DEET, the repellency of pyrethrum diminished significantly.   The next round of testing then looked at mosquitoes with a mutated *Orco* gene which has shown to lead to a lack of DEET repellency, the previously used positive control repellent. The *orco* mosquitoes were shown to be unrepelled or slightly repelled at two different concentrations of pyrethrum which successfully repelled wild-type mosquitoes of the same species. This would indicate that pyrethrum repellency is *Orco*-dependent.

c. Study 3

A second study looking at the efficacy of pyrethrum repellency against pyrethroid resistant mosquitoes conducted by Duchon et al. (2009) looked at the *kdr* mutation in *Anopheles gambiae*. For this test, both pyrethrum-based products and synthetic pyrethroids were used. To begin the study, a dose response relationship was established by topical applications to estimate the discriminating dosage. Once the dosages were understood, a WHO cone test was conducted on treated bed nets to understand knockdown and mortality, followed by a tunnel test with bed nets to evaluate mortality and feeding inhibition (repellency).

In the initial tarsal contact assay, pyrethrum and permethrin acted at a similar range against susceptible mosquitoes, with deltamethrin acting at a much lower concentration. All three actives resulted in 100% mortality against the susceptible strain, with 90%, 80%, and 72% mortality respectively against pyrethrum, deltamethrin, and permethrin against the *kdr* mosquitoes. This is very interesting in the fact that the *kdr* mutation did not greatly affect the pyrethrum mortality like it did the synthetic pyrethroids. The authors conclude that this could be a promising use for pyrethrum in bed net treatments against Malaria vectors, especially when the presence of *kdr* pyrethroid resistance is in the population.

d. Study 4

While most of the study conducted by Yan et al. (2021) is like those done in previous studies, the importance here is the team focused on the Asian tiger mosquito *Aedes albopictus*. For this they looked at both repellency and EAG responses when exposed to the six individual pyrethrin components. In the repellency portion, a standard hand-in-cage assay was conducted. This showed that *Ae. albopictus* were repelled by four of the esters: pyrethrins I, and II, cinerin II, and jasmolin II. The team then moved on to the EAG and elicited responses to the previous four, with the addition of cinerin I. This study further cements the proof that pyrethrum evokes an olfactory response in mosquitoes, as well as a spatial repellency response, by showing it in a previously unstudied mosquito for these responses.

IV. Cockroaches and Ants

While the public might not often associate crawling insects with repellency, it is becoming an emerging trend in markets where more natural offerings are desired. Repellents are now being used to prevent insects like cockroaches and ants from entering living spaces by treating access points such as thresholds and utility entry points, instead of simply spraying to kill them. There is not, however, a lot of evidence supporting this as a successful option for effectively treating for these pests.

a. Study 5

In a study conducted by Eberling et al. (1966) looking at the influence of repellency on the efficacy of blatticides, it was discovered that cockroaches could modify their behavior after being exposed. While this older study mainly focused on insecticides no-longer in use, there were still some interesting findings related to repellency and pyrethrum.

For this study a series of devices and procedures were developed to help determine the behavior of German cockroaches, *Blattella germanica*, when given a choice of entering or avoiding treated areas. This included a full mock-up kitchen designed to be able to monitor cockroach movement. A major takeaway from this test showed that repellency by the treatments was largely driven by how stressed the cockroaches were. If the cockroaches were placed into the kitchen and given the ability to enter the treated areas that served as harborages, they almost always did immediately. When the harborages were blocked for an initially period allowing the cockroaches to acclimate to the area, they were less likely to enter the treated spaces once allowed. The availability of food and water and the presence of untreated harborages also impacted the frequency that the cockroaches would enter the treated spaces.

From there, Eberling and team designed “choice boxes,” which allowed them to observe the behavior of cockroaches when given the choice of entering a dark, but treated, area or remaining in a lighted, untreated area. Food and water were also supplied on the untreated side. This again showed the cockroaches almost exclusively avoided the treated areas and instead stayed in the lighted side of the box.

While this may at face value look like a positive for using pyrethrum as a repellent, the downside as shown is this study is the ability of cockroaches to modify their behavior and inhabit areas in the vicinity that are untreated. In the end what usually takes place is the cockroaches expand their range to areas they might not have occupied previously, instead of being repelled from the home.

b. Study 6

This next study, which looked at the repellency and efficacy of various insecticides against Argentine ants, was conducted by Knight and Rust (1990). To evaluate these insecticides, they field collected ants and brought them back into a lab setting. They then constructed artificial colonies that consisted of two petri dishes connected by tubing. One dish contained the nest, while the other was a feeding arena. The ants would then move between the petri dishes via the tubing to collect food and bring it back to the nest. Natural movement of the ants was low, so an artificial trail pheromone was introduced to increase movement between the dishes. After one week of acclimation, a section of the tubing was replaced with a treated section. Ants were then monitored and any ant that passed through the treated section was counted. The number knocked down was viewed, and any mortality was recorded.

To examine repellency differences between the formulas tested the team ranked the treatments from least to most repellent, and then divided them into three groups of high, medium, and low. The same was done for efficacy regarding knockdown and mortality. The two pyrethrin based formulas were both ranked as medium for repellency. One was a wettable powder, and the other was an emulsifiable concentrate.

V. Conclusion and Discussion

Pyrethrum has long been known as an effective and relatively safe insecticide and repellent. Much effort has gone into studying the repellent effects in mosquitoes, and much is known about the mechanisms of repellency involved. This encompasses two of the most prevalent disease vector genera in *Aedes* and *Anopheles*. Liu et al. (2021) showed that repellency in *Ae. aegypti* is dependent on the olfaction system, specifically the antenna, based on responses from an EAG. Maxillary palps showed no such response when tested. This led to the discovery that when odorant receptor AaOr31 was silenced it resulted in up to a 60% decrease in repellency. This studly ultimately showed a dual-target mechanism for repellency by pyrethrum through the olfactory system, and sodium ion channel disruption. In Yan et al. (2021) the authors also looked at EAG responses to pyrethrin components as well as behavior responses. This study, however, focused on *Ae. albopictus.* While this paper did not look at individual receptors, it did prove that Asian tiger mosquito repellency to pyrethrum is also a dual-target mechanism by combing the two experiments. Another study, Bandason (2018), went a step further and started looking at what happens when you run hand-in-cage tests against two different mosquito strains with known resistance. One with pyrethroid resistance (Puerto Rico), and one with DEET resistance (*Orco)*. This showed that when mosquitoes are *kdr* resistant to pyrethroids, they still are repelled at the normal level to DEET. However, when mosquitoes with a mutated *Orco* gene were tested against pyrethrum, they were not repelled. This would indicate that pyrethrum repellency is also *Orco* dependent. One final study in the mosquito portion of this review, Duchon et al. (2009), looked at *kdr* in *An. gambiae*. This study focused on tarsal contact by looking at bed net treatments. While the two synthetic pyrethroids used in the study showed a significant drop in mortality against the *kdr* mosquitoes compared to the susceptible strain, the pyrethrum treatment did not. This is particularly interesting and should lead to more studies against different mechanisms of resistance, as *kdr* is just one of many.

Where the literature seems to be lacking is regarding repelling crawling insects effectively, opening an avenue for further studies. The studies reviewed did show pyrethrum may be good at keeping a cockroach out of a treated space, they often just move to the next available untreated area. This could drive potential resistance issues from constant sub-lethal exposure in an insect already known for becoming resistant to pyrethrins and their synthetic versions, pyrethroids. A more practical approach would be to utilize more tools from an IPM approach to create a push-pull scenario by using pyrethrum sprays to push cockroaches from an area and pull them into a toxic bait station. There is also opportunity to look at the effectiveness of treatment location by pyrethrum sprays in concert with baits. Are certain entry points or construction features more important to treat? Where is the best location for bait stations when combined with sprays? Ants also seem to be another area available for further research. Argentine ants, at minimum, only show a medium level of repellency against pyrethrins, but not much work has been done on other species of interest. One would think that carpenter ants could be a point of interest for a study like this. While structural pests often need more thorough treatment due to the risk of damage, there could still be potential application as a barrier treatment, especially if the photosensitivity of pyrethrum could be addressed allowing for a longer availability of the active. One option could be microencapsulation to allow for a release of active over time. It would also be interesting to study the repellency effects of pyrethrum on an indoor nesting ant, like the Pharaoh ant, *Monomorium pharaonic*, to see the impact one could have by treating potential nesting sites.

In summary there has been a lot of good work in the field of pyrethrum repellency against mosquitoes. Genes and receptors have been identified as vital to repellency, allowing for further study and potential development of actives or products to improve repellency. It has also been shown that pyrethrum has potential to combat pyrethroid resistance in certain scenarios, namely in bed net situations. While not as much attention has been paid to urban pests, such as ants and cockroaches, some good preliminary work has been done, opening avenues to explore further and see if successful strategies can be discovered.

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