Thyme essential oil in corn starch particles combats Aedes aegypti larvae

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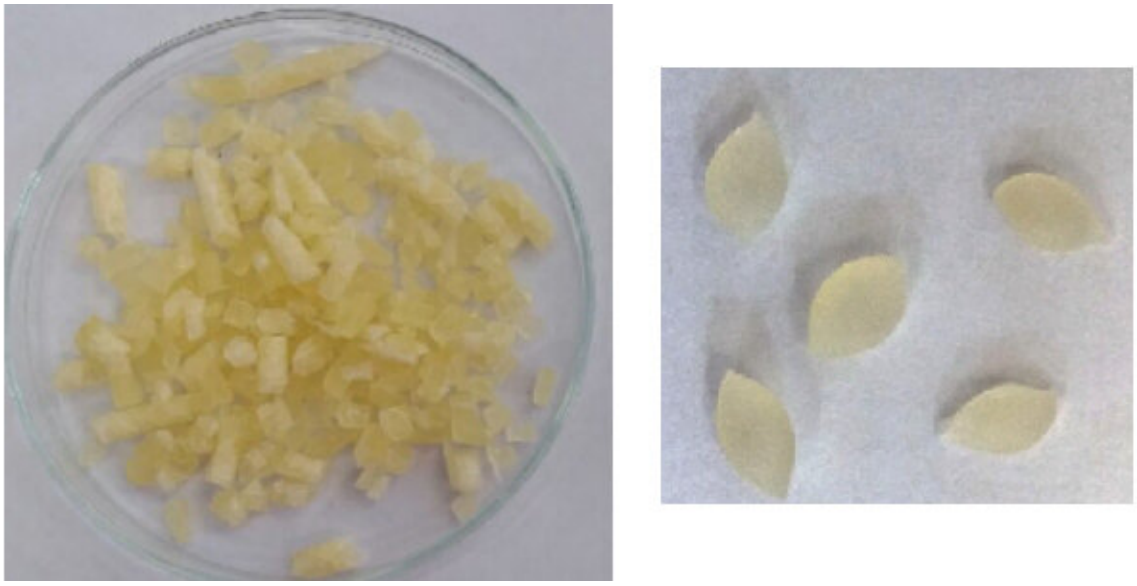

A system created in Brazil using cheap, biodegradable materials permits controlled release of larvicide and can be used in small amounts of water. Credit: Ana Silvia Prata

Corn starch, an abundant, cheap and biodegradable raw material, is the basis for a novel larvicide developed by researchers at the University of Campinas (UNICAMP) in São Paulo State, Brazil. The material is used in microcapsules for storage and controlled release of active compounds to kill larvae of *Aedes aegypti*, the mosquito that transmits dengue, zika, yellow fever and chikungunya.

A patent application has been filed via UNICAMP's innovation agency (Inova). The methodology is described in an article published in *Industrial Crops and Products*.

The research and development was supported by São Paulo Research Foundation—FAPESP. The principal investigator was Ana Silvia Prata, a professor in the university's Food Engineering School (FEA-UNICAMP).

The published paper describes a biodegradable controlled release system using thymol as the microencapsulated larvicide. Thymol, a key ingredient of essential oil derived from thyme (*Thymus vulgaris*), a plant widely used as a pharmaceutical and therapeutic agent, is also biodegradable and not harmful to humans in the concentration used by the researchers.

"We succeeded in obtaining a particle that behaves exactly like eggs laid by *A. aegypti*," Prata said. "While the environment is dry, it remains inert and keeps the active agent protected. As soon as it comes into contact with water, it begins to swell up and release the larvicide. After three days, when the eggs hatch and the larval stage begins, the particle starts to release lethal quantities of the active principle into the water."

The researchers set out to develop a system for controlled release of larvicide in backyards, gardens, utensils and other household objects that may contain water, including bottles, potted plants, old tires and rubble, all of which are breeding grounds for *A. aegypti*.

According to Prata, public health authorities in Brazil tend to focus on treating water tanks and other large reservoirs with larvicide, but epidemiological studies show that small containers account for 50% of mosquito breeding grounds.

"This is a low-cost larvicide, so the government can produce the particles and distribute them to the public for placement around the home where rainwater accumulates. The idea is to supplement educational and awareness campaigns against dengue," she said.

In tests conducted at UNICAMP, the particles remained functional during approximately five rain cycles. After first contact with water, they released only 20% of the thymol. "In one of our tests, we let the material dry and then rehydrated it, after which the particles again released the larvicide normally," Prata said.

She added that thymol, the key active ingredient in thyme essential oil, is an anti-microbial and blocks the proliferation of microorganisms in water containers, preventing rapid spoilage of the particles after they become wet.

Production method

The life cycle of *A. aegypti* comprises four stages: egg, larva, pupa, and adult mosquito. The development stage varies from five to ten days, becoming shorter as temperatures rise. The larval stage takes place in water and is considered strategic as far as combating the proliferation of the mosquito is concerned.

"Based on this information, we thought about how to produce the particle. One of our collaborators, Johan Ubbink [California Polytechnic State University, USA], suggested extrusion. This is the method used to produce breakfast cereals such as cornflakes and savory corn snacks," Prata said.

The method consists of heating wet starch and forcing it through a small hole. The heat and pressure normally make the material expand after exiting the hole.

"We adapted the process by using a lower temperature and slower screw speed to avoid expansion of the material. If it swelled, the particle would dissolve too quickly on contact with water, releasing the active principle all at once," Prata said.

Another challenge for the group was finding the right composition for the raw material. As Prata explained, starch from corn, wheat or any other plant consists mostly of amylose and amylopectin in varying proportions. The quantity of each substance determines properties such as viscosity and structure, influencing the material's integrity when in contact with water.

"We tested formulations with proportions of amylose ranging from 1.8% to 76%, and in each case evaluated leaching [loss of solids by solubilization] and swelling in an aquatic environment," she said.

At the same time as they evaluated these two aspects of the particle to dose the quantity of thyme essential oil released as a function of the water contact time, the researchers also observed the larvicidal activity of the active principle. This test consisted of measuring the "lethal concentration" (LC) required to kill 99% of the larvae, a parameter known as LC99.

"The LC99 of nonencapsulated thyme essential oil is approximately 70 micrograms per milliliter [μg/ml]. When we put the oil into the particle, it fell to 31 μg/ml. In other words, our controlled release system increased its larvicidal action," Prata said.

Even so, the natural compound's LC99 remained far lower than that of synthetic agents such as temephos. The main advantage of using thyme essential oil, according to Prata, is that the mosquito is unlikely to develop resistance to it because of its complex chemical composition, which includes other active molecules besides thymol.

The group also tested the method using paracress (Acmella oleracea) as a larvicide. The result was similar, but the cost was approximately 15 times that of thymol.

"Thyme essential oil is plentiful and commercially available. It corresponds to only 5% of the particle's composition.

The rest is corn starch, which is very cheap. We therefore consider the technique to be easily scalable," Prata said.

The group is currently studying the possibility of using the same particles to encapsulate nitrogen-fixing bacteria, which assist plant growth. Theoretically, the material could reduce the amount of fertilizer needed in agriculture. "We plan to test this theory in a future project," Prata said.

More information: Juliana Dias Maia et al, Improved activity of thyme essential oil (Thymus vulgaris) against Aedes aegypti larvae using a biodegradable controlled release system, *Industrial Crops and Products* (2019). DOI: 10.1016/j.indcrop.2019.03.040

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