

Scientists decode how North Bengal's dengue mosquitoes are dodging insecticides. G.S. Mudur reports

Never-ending contest



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From his lab in Siliguri, entomologist Dhiraj Saha has found a possible way to counter an old problem in North Bengal's long struggle against *Aedes aegypti* mosquitoes — block the enzymes that help them resist insecticides. But Saha suspects that respite will be short-lived because, as he puts it, humans and mosquitoes are engaged in “a never-ending contest”.

For about a decade, Saha has been tracking the rise of insecticide resistance in *Aedes* mosquitoes. He is focused on *Aedes* because this species plays a major role in spreading dengue, a viral infection whose estimated cases have increased 25-fold over the past two decades. Since 2015, his studies in the districts of Darjeeling, Jalpaiguri, Alipurduar, Cooch Behar and Uttar Dinajpur have revealed growing resistance to different classes of insecticides in the *Aedes aegypti* — from the older organochlorine compound DDT to pyrethroids such as deltamethrin and permethrin to organophosphates such as malathion and temephos.

Now, Saha and his colleagues have probed the biological machinery that allows *Aedes aegypti* mosquitoes to evade malathion and temephos. They found that mosquitoes resistant to these organophosphates have unusually elevated levels of detoxification enzymes called carboxylesterases. “The greater the amount of enzyme a mosquito produces, the faster it is able to eliminate the insecticide from its body,” Saha, professor of zoology at the University of North Bengal, tells *The Telegraph*.

To verify whether the elevated carboxylesterases were driving resistance, the researchers exposed the insecticide-resistant mosquitoes to triphenyl phosphate (TPP), a chemical that blocks carboxylesterase. In lab experiments, the insecticides killed less than 85 per cent of

resistant mosquitoes, but achieved 100 per cent mortality when the mosquitoes were pre-exposed to TPP.

The finding points a way to restore the effectiveness of organophosphates against resistant mosquitoes. “We’re hoping these observations will help guide more evidence-based control strategies for dengue mosquitoes,” Saha says.

One option would be for public health authorities to incorporate TPP alongside malathion and temephos in control efforts. “Our lab studies suggest that exposure to TPP

jit Das set out to investigate the mechanisms at play in resistant *Aedes aegypti*. The clues emerged fast. They found that almost all mosquitoes resistant to organophosphates sampled from Darjeeling, Jalpaiguri and Alipurduar had significantly higher levels of carboxylesterases. Mosquitoes sampled in Cooch Behar and Uttar Dinajpur showed lower levels of resistance to these organophosphates.

The mosquito's life stage also influenced the levels of enzymes. In resistant *Aedes* larvae, the gene CCE-

how malathion and temephos interact with carboxylesterases. The simulations, together with their laboratory experiments, strengthened the link between the enzymes and insecticide resistance: resistant mosquitoes had higher carboxylesterase activity, the genes involved in producing the enzymes were more active, and the enzymes could interact with the insecticides in ways consistent with their role in detoxification.

But turning that lab observation into an effective and sustainable field intervention is a separate challenge. Given how quickly mosquitoes have evolved resistance to one insecticide after another over the past decades, Saha said, it is possible they will eventually evade TPP too. “With insecticides, we’re engaged in a never-ending contest against mosquitoes,” he said. “Our best strategy would be to lower dependence on synthetic insecticides and adopt long-term sustainable approaches that seek to keep mosquito populations as low as possible by decreasing their source populations.”

Reducing dependence on insecticides would mean habitat control to curb *Aedes aegypti* breeding.

A separate study by Saha and his colleagues earlier this year had pointed to one such opportunity: discarded tyres. Their study found mosquito larvae in 605 of 1,104 discarded tyres surveyed across 34 sites along 200km of highways in the five districts. And 63 per cent of the discarded tyres were within 50 metres of homes. “We cannot keep chasing mosquitoes with one insecticide after another, while allowing them to breed unchecked,” Saha said. “The more we reduce their breeding sources, the less dependent we become on insecticides.”

Other faculty and research scholars who contributed to the study include Prapti Das, Subarna Thakur, Abhirup Saha, Subhajit Ghosh and Abhinna Tamang.



SPOTTED: An earlier study by Saha found that discarded tyres were perfect breeding grounds for the *Aedes aegypti* (top)

will restore the mosquitoes’ vulnerability to the insecticides,” Saha says.

Studies on insecticide-resistant mosquitoes elsewhere in the world since the 1970s have established that resistance can emerge through different detoxification enzymes — some mosquitoes use P450 enzymes, others use glutathione S-transferases, still others use the carboxylesterases. Which enzymes are involved and how strongly they are produced can vary depending on the mosquito species, the insecticides they have been exposed to and local environmental conditions.

Saha and research scholar Subha-

ae3a was expressed at levels between 14 and 39 times those seen in susceptible mosquitoes. In *Aedes* adults, another gene CCEae6a was overexpressed 12 to 34 times. The distinction is relevant because public health authorities typically spray temephos in water where mosquito larvae grow, but use malathion against adult flying mosquitoes. The findings suggest that different parts of the detoxification machinery become more active at different stages of its life.

The scientists then used molecular docking studies — 3D computer simulations that predict how molecules may fit together — to examine