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Commentary

Fly ash-based *Bacillus thuringiensis israelensis* formulation: An ecofriendly approach

Vectors play an important role in disease transmission, globally. Despite various advancements in vector management, mosquitoes are still prime vectors of dreadful diseases those influence human health and economy as well. Insecticides have been the most preferred and commonly used tool in the vector management because of their rapid action and visible effects. Although several insecticides in different formulations are in existence, their usage over the years has arisen several problems such as reduced response by vectors in the form of resistance phenomenon and their negative impact on the environment.

Amongst various vector management options, biopesticide is a comparatively safer way. Biopesticides based on microorganisms can play an alternative strategy in crop protection due to safety to human beings and non-target organisms[1]. Although biopesticides represent only 2.89 per cent of the overall pesticide market in India[2], its demand is rising steadily in all parts of the world[3]. An increased concern of biopesticides as an effective tool for mosquito control has been witnessed[4]. Amongst the various biopesticides, microbial pesticides represent the major component. Bacterial biopesticides are comparatively cheaper than the other methods of pest bioregulation[3]. It is evident that *Bacillus thuringiensis* is one of the most promising biopesticides, representing 70 per cent of the global biopesticide market[2]. *Bacillus* species, *B. thuringiensis,* is known for its property to produce proteins which are structurally distinct and acts as pesticidal toxins against pests of agriculture and medical importance; those are coded for by several *cry* genes[5].

The first commercial *Bt* product was produced in 1938 in France[6]. To date, over 100 *B. thuringiensis*-based bioinsecticides have been developed, which are mostly used against lepidopteran, dipteran and coleopteran larvae[3]. *Bt* is effective against more than 150 insect pests. Because of their high specificity and safety in the environment, *B. thuringiensis* and Cry proteins are efficient, safe and sustainable alternatives to chemical pesticides for the control of insect pests[7,8]. Amongst 34 recognized subspecies of *B. thuringiensis,* subspecies *kurstaki* (lepidopteran specific), *israelensis* (dipteran specific, mainly mosquitoes and blackflies) and *tenebrionis* (targeted to Colorado potato beetle) are most widely explored[9].

Biopesticides in several formulations such as dusts, powders for seed dressing, granules (GR), micro-GR, water-dispersible GR and wettable powders (WP); suspension concentrates, oil dispersions, suspo-emulsions, capsule suspensions; ultra-low volume formulations are in existence[10,11]. In India, only 12 types of biopesticides have been registered under the Insecticide Act, 1968[3]. *Bacillus sphaericus* Neide (*Bs*) and *B. thuringiensis* serovar *israelensis (Bti)* are proven potential mosquito larvicides that stand effective in many operational levels under field conditions. These have numerous advantages such as safety for humans and other non-target organisms, minimal residues in the treated habitats, safe to most other natural enemies and increased biodiversity in aquatic ecosystems[4]. *Bti* has been successfully advocated for the management of mosquitoes since the last two decades, and its formulations are highly efficient against all three mosquito genera *Anopheles*, *Aedes* and *Culex*[12]. It has also been documented that *B. sphaericus* recycles in the field conditions and exhibits prolonged larvicidal activity, mainly against *Culex* and a few *Aedes* species, whereas *Bti* shows a wide spectrum of activities against all three species[4]. However, repeated application of *B. sphaericus* in the same habitat has evolved resistance in larvae[13]. During field experimentation, efficacy of different *Bti* formulations lasted for 2-7 days against *An. culicifacies* in freshwater pools and *Cx. quinquefasciatus* in polluted pools, 2-14 days against *An. stephensi* in tanks and drains and 7-28 days against *Ae. aegypti* in desert coolers and industrial scrapes[13].

Both the bacterial formulations have been extensively studied under different geographical regions under variety of habitats. The efficacy of the of *Bt* depends on various bioenvironmental factors and the type of formulation. Formulation can affect the persistence of toxicity, site of contamination and choice of application method, as well as ultraviolet radiation, agitation, sedimentation, water quality, pollutants, *p*H, temperature, target host and microbial competition[14]. Since these bacteria are safe for animals and environment and cause no health risk to humans, several formulations have been produced to control many species of mosquitoes[4]. *Bti* formulation such as aqueous suspension, WP and GR have been tested for efficacy in the field[13]. *Bti* in briquettes or pellets form can address the problem of persistence[14], whereas floating GR are better options for surface feeders. Effective formulations for control of different mosquitoes have been developed. Some formulations are slow release controlled formulation, mixture of chemical and biological agents, sprayed-dried powder as tablet and floating bait[14]. With the advent of nanotechnology in biological sciences, optimized controlled release formulations of *Bti* in the form of nanoemulsion, nanosuspension, nanocapsule suspension, *etc*. are new hope in bringing better biological effects[15-17].

An effective, broad-spectrum, low-cost, user-friendly and readily available *Bti* formulation is needed. The cost to grow and produce *Bti* in highly refined laboratory bacterial culture medium is high[18]. For economic *Bt* production, cheaper raw material is essential. Several low-cost raw materials have been utilized for production of *Bt* biopesticides in India. Agro-industrial remains are simple, cheaper and effective bioresource for fermentation producing bacterial toxins targeting mosquito vectors[4]. Several agro byproducts and waste materials such as bird feathers, dried animal blood, fish meal and coconut cake soybean have been documented as alternate source for culture medium for *Bt* production[18-20]. All these raw materials are rich in nutrient sources (carbohydrate and proteins) and lead to the production of bacterial biopesticides *Bs* and *Bti*[21,22].

Charcoal and plaster of Paris are some of the common carrier material for *Bt* formulations. Tamilselvam *et al*[23] in this issue have reported a new carrier material fly ash as an alternative to the common carrier powder-based *Bti* formulation. Use of fly ash as a carrier in insecticides is generally known. Enormous amount of fly ash which is generated as solid waste in thermal power plants is available in India. Coal-based thermal power plants are the chief source of power generation[24]. During 2014-2015, 184.14 million tons of fly ash was generated[25]. The disposal of the substantial amount of solid waste from thermal plants is becoming a prime concern to human health. Fine particles of fly ash accumulated in the lungs for long period act as cumulative poisons and residual silica (40-73%) may cause silicosis, whereas heavy metals can exhibit toxicity[26]. Prolonged exposure to toxic metals in coal ash can cause several types of diseases[27].

There is a vast gap between generation and utilization of fly ash among the countries. In developed countries such as Germany, 80 per cent of the fly ash generated is being utilized, whereas it is only three per cent in India[26]. Utilization of fly ash in the biopesticide formulations can contribute to its proper utilization thereby decreasing environmental pollution. Fly ash-based *Bti* formulation has been evaluated in field against *Culex* mosquito in natural ecosystem[28] and found effective in reducing larval population, regardless of habitats.

The Indian fly ash is alkaline, facilitates to improve soil quality, maintains porous structure of soil, provides micronutrients and improves the fertility[24]. Fly ash has earlier been effectively used as carrier in production of biofertilizers and biopesticides[29]. A range of fly ash-based *Bti* formulations have been evaluated against all three vectors[23], to arrive at the most effective one. Indian fly ash has very low radioactivity and heavy metal count[30]. Analysis of the shortlisted formulation by Tamilselvam *et al*[23] also revealed the presence of only micro- and macronutrients and absence of any kind of heavy metals. This formulation has also shown safety against non-target organisms and mammalian systems. Further investigation on fly ash from different sources also needs to be done in detail. Although this formulation has potential to replace existing carrier material in biopesticides, an in-depth study on several aspects such as long-term toxicological analysis, its persistence in different habitats, storage stability and its economic needs is to be conducted.

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