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Original article

# The efficacy of propylene glycol alginate (PGA), a food additive, in controlling *Haemaphysalis longicornis* ticks

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ABSTRACT

Ticks and tick-borne pathogens threaten the health of both domestic animals and humans, and are associated with a high economic burden in many countries. Tick control can be achieved with chemical acaricides, but issues remain regarding their safety as well as emerging tick resistance. Propylene glycol alginate (PGA) is a food additive commonly used in China. It has been used to kill whiteflies in agriculture as an environmentally friendly insecticide. The aims of this study were to (i) explore the efficacy of PGA to kill *Haemaphysalis longicornis* ticks and (ii) assess the potential to develop a new tick control acaricide containing PGA and a reduced amount of synthetic pyrethroid. Beta-cypermethrin was chosen as the reference pyrethroid in this study. PGA, beta-cypermethrin and mixes (PGA and beta-cypermethrin formulated in proportions of A = 2:1, B = 1:1, and C = 1:2) were compared for efficacy to kill larval and adult *H. longicornis* ticks. Overall, we found no statistically significant differences in the killing efficacy of PGA as compared to beta-cypermethrin across examined time-points post-tick exposure. At 24 h post-tick exposure, similar killing efficacy for *H. longicornis* larvae was recorded for beta-cypermethrin alone, PGA alone, and mixed formulations B and C. Mixed formulation C had the strongest killing effect when compared to PGA alone or mixed formulation B. Similar outcomes were observed in experiments with adult *H. longicornis* ticks. Based on these findings, we propose that PGA can be useful as a tick control acaricide, either as a single active ingredient or formulated together with a pyrethroid.

## 1. Introduction

Ticks playa significant role worldwide in the transmission of various pathogens, including those causing Lyme disease, severe fever with thrombocytopenia syndrome (SFTS), tick-borne relapsing fever, Q fever, and north Asian tick-borne rickettsia disease (Brites-Neto et al., 2015; Mayne, 2014). Moreover, tick infestation of livestock can result in economic burdens (Wei-jun et al., 2014). To counter this threat, a wide range of pesticide-active ingredients, including lindane, dichlorodiphenyltrichloroethane (DDT), amitraz, stirofos, dursban, fluazuron, fipronil, and synthetic pyrethroids, have been formulated to control ticks on and off hosts (Guang-peng et al., 2011; Stafford, 2007). Although these acaricides are effective in controlling ticks, there are health safety concerns (Alavanja et al., 2004; Rosas and Eskenazi, 2008), environmental concerns, and reports of emerging tick resistance (Heath and Levot, 2015). Hence, it is highly desirable to develop novel acaricides with better safety profiles.

Propylene glycol alginate (PGA), made from the kelp plant or from certain kinds of algae, was approved for use as a food thickener in the National Standard GB2760 of China in 1988 (Wei-dong and Li-ya, 1998). However, 0.12% PGA also can serve as an effective, insecticide to control *Trialeurodes vaporariorum* whiteflies, based on the certification of the United States Environmental Protection Agency (USEPA, EST.NO.074533-CA-001). In this context, PGA acts via a physical mechanism. It occludes the cuticle/ spiracles of pests so that no respiration can take place and that the pests eventually die of oxygen deficit. Based on this physical mechanism, and with no apparent side-effects documented to date, PGA was exempted from food additive tolerance requirements in the European Union and the United States. However, the potential for using PGA against ticks has been unclear.

Prior to this study, a pilot experiment targeting larval *Haemaphysalis longicornis* ticks was conducted to explore the potential lethal activity for using PGA, and mixed formulations of PGA and beta-cypermethrin, against ticks (Yu-yan et al., 2017). Beta-cypermethrin is a synthetic

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pyrethroid pesticide. It is widely used across the world, and acclaimed for its high efficacy and low toxicity for humans and other warm-blooded animals. Recently, with the development of resistance in ticks and rising expectations of even lower toxicity, scientists continue to seek alternatives for beta-cypermethrin (Hai-yan et al., 2015). In this study, we use beta-cypermethrin as the reference to assess the efficacy of PGA alone and mixed formulations of PGA and beta-cypermethrin in controlling ticks. We chose *H. longicornis* for these studies because of its importance in China as a livestock pest and potential vector of SFTS virus (Luo et al., 2015). The aims of this study were to (i) explore the efficacy of PGA to kill *H. longicornis* ticks and (ii) assess the potential to develop a new tick control acaricide containing PGA and a reduced amount of synthetic pyrethroid.

## 2. Materials and methods

### 2.1. Bioassays with H. longicornis larvae

#### 2.1.1. Tick collections

Thirty engorged *H. longicornis* females were collected from naturally infested cattle in Daishan city, Zhejiang province, China. Each fed tick was placed in a polyethylene tube with a ventilate silica gel stopper and transported to the Vector Laboratory of the Zhejiang Provincial Center for Disease Control and Prevention (Zhejiang CDC). The female ticks were incubated at 27 ± 1℃ and high humidity (> 80%) under a 12-h light/dark cycle until they oviposited (Edmeia et al., 2011). Eggs from one randomly chosen female were selected.

#### 2.1.2. Pesticide to be tested

Beta-cypermethrin emulsifiable solution (5%) and PGA were obtained from Jiangsu Gongcheng chemical co., LTD, Nantong city, China and Zhengzhou Siyang chemical co., LTD, Zhengzhou city, China, respectively. Progressive dilution series of the stock solutions were made using distilled water to provide lower concentrations. The range of concentrations used included both those recommended by the manufacturers (3 mg/L for PGA, and 450 mg/L for beta-cypermethrin) and concentrations known to be highly lethal (LC90) to *H. longicornis* ticks from our pilot studies (unpublished data, 5.75 mg/L for PGA, and 388.78 mg/L for beta-cypermethrin). As shown in Table 1, five formulations were tested. These included PGA alone, beta-cypermethrin alone, mixed formulation A (PGA: beta-cypermethrin = 2:1), mixed formulation B (PGA: beta-cypermethrin = 1:1), and mixed formulation C (PGA: beta-cypermethrin = 1:2). PGA was prepared using dilution ratios of 1:100, 1:200, 1:300, 1:400, and 1:500, while the other formulations were prepared using dilution ratios at 1:50, 1:100, 1:150, and 1:200. Three replicates were conducted for each formulation or control group (distilled water), for a total of 66 bioassays. The sums of ticks from all three replicates from each formulation were used in the analysis.

#### 2.1.3. Experimental procedure

Bioassays with larvae were carried out in a temperature-controlled chamber at 27 ± 1℃ with relative air humidity > 80% and a 12-h light/dark cycle (Edmeia et al., 2011). The bioassay arena consisted of a petri dish with a filter paper (10 cm in diameter) on the bottom and double-sided adhesive tape over the edges of the filter paper and the dish to prevent ticks from escaping. Each bioassay replicate included 10 unfed larvae that were 14–21 d old. Following introduction of larvae onto the filter paper, the petri dish was sprayed with 600 µl of a specific test formulation or the distilled water control, after which it was immediately sealed. To avoid contamination, each test formulation was prepared and sprayed in a separate room. Mortality was assessed using a stereo microscope after 0.5, 1, 24, and 48 h of exposure including gentle prodding of the ticks to induce movement, according to the recommendations of the Food and Agriculture Organization of the United Nations (FAO) (Edmeia et al., 2011; FAO, 2004; Yu-yan et al., 2017).

### 2.2. Bioassays with H. longicornis adults

Unfed male and female *H. longicornis* ticks were collected from vegetation in Daishan city (North), Linhai city (East), Lishui city (South), and Quzhou city (West), aimed at a full geographic coverage of the Zhejiang province, and then transported to the Vector Laboratory of Zhejiang CDC. They were then maintained as described above for the larval ticks, with mixed females and males, prior to use in bioassays. The preparation of test formulations, and bioassay replication, followed the same procedures as those described for the larval bioassays. In contrast to the bioassay procedures for larvae, 10 randomly chosen adult *H. longicornis* ticks were placed in an open-top Petri dish with filter paper. The Petri dish was then placed on top of a beaker that sat in a container half-filled with water to prevent escapes. Each petri dish was then sprayed with 600 µl of a specific test formulation or distilled

**Table 1**
Components and dilution ratios of the acaricide formulations examined.

| Acaricide formulation | Dilution ratio | Concentration(mg/L) | AI per unit area (g/ha) |
|---|---|---|---|
| Propylene glycol alginate (PGA) | 1:100 | 12 | 9.17 |
| | 1:200 | 6 | 4.59 |
| | 1:300 | 4 | 3.06 |
| | 1:400 | 3 | 2.29 |
| | 1:500 | 2.4 | 1.83 |
| Beta-cypermethrin | 1:50 | 1000 | 764.33 |
| | 1:100 | 500 | 382.17 |
| | 1:150 | 333 | 254.78 |
| | 1:200 | 250 | 191.08 |
| Mixed formulation A: beta-cypermethrin: PGA = 1:2 | 1:50 | 333 + 16[a+b] | 254.78 + 12.23 [a+b] |
| | 1:100 | 167 + 8 [a+b] | 127.78 + 6.12 [a+b] |
| | 1:150 | 111 + 5.3 [a+b] | 84.93 + 4.05 [a+b] |
| | 1:200 | 84 + 4 [a+b] | 64.27 + 3.06 [a+b] |
| Mixed formulation B: beta-cypermethrin : PGA = 1:1 | 1:50 | 500 + 12 [a+b] | 382.17 + 9.17 [a+b] |
| | 1:100 | 250 + 6 [a+b] | 191.08 + 4.59 [a+b] |
| | 1:150 | 167 + 4 [a+b] | 127.78 + 3.06 [a+b] |
| | 1:200 | 125 + 3 [a+b] | 95.54 + 2.29 [a+b] |
| Mixed formulation C: beta-cypermethrin : PGA = 2:1 | 1:50 | 666 + 8 [a+b] | 509.56 + 6.12 [a+b] |
| | 1:100 | 333 + 4 [a+b] | 254.78 + 3.06 [a+b] |
| | 1:150 | 222 + 3 [a+b] | 169.85 + 2.29 [a+b] |
| | 1:200 | 167 + 2 [a+b] | 127.78 + 1.53 [a+b] |

[a]Concentration of beta-cypermethrin.
[b]Concentration of PGA.