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Short communication

Aphidicidal activity of a novel botanical insecticide made by alkalization of bamboo tar

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ABSTRACT

Bamboo tar is a water-insoluble semi-solid waste generated from bamboo charcoal processing, and its increasing accumulation threatens environment and sustainability of the charcoal industry that enhances the economic value of bamboo forestry in southern China. Here we converted bamboo tar to a completely water-soluble sodium salt through full alkalization with NaOH and evaluated its insecticidal activity against the cotton aphid *Aphis gossypii*, the soybean aphid *Aphis glycines* and the green peach aphid *Myzus persicae* in laboratory bioassays and field trials. The tar salt LC_{50}s against the three global aphid pests decreased to 35–93 μg/ml on day 7 from 246 to 486 μg/ml on day 3. In three field trials, the tar salt at the application rates of 338–675 g/ha controlled the aphid pests as efficaciously as but more persistently than pymetrozine (a synthetic aphidicide) at the recommended rate. Our results highlight an easy approach to converting bamboo tar to a promising botanical aphidicide but extensive further work to determine its toxicity and environmental and crop safety is needed.

Aphids (Hemiptera: Aphididae) are cosmopolitan insect pests infesting many crops and economically important plants, causing serious damages by sucking plant juice with piercing mouthparts and transmitting plant viruses in the feeding process (Blackman and Eastop, 2000). Long-term reliance on synthetic chemical insecticides for aphid control has resulted in high resistance among many aphid species and environmental problems (Margaritopoulos et al., 2007; Ragsdale et al., 2007; Bhatia et al., 2011; Silvie et al., 2013). The incidences of increasing insect resistance to the insecticides with active ingredients of singular synthetic chemicals shortens their market longevity, facilitating a search for potential phytochemicals that can be exploited for insect pest control (Gao et al., 2004; Song and Swinton, 2009; Dayan et al., 2009).

Bamboo tar is a water-insoluble semi-solid waste byproduct from the bamboo charcoal industry, which has been developing to enhance the economic value of bamboo forestry in Southern China. The charcoal making relies upon bamboo carbonization at 700–750 °C, and the process annually generates thousands of tones of bamboo tar (Lu and Zhou, 2009). This waste has been shown to comprise more than 90 compounds, including homologues and derivates of phenols (43.63%), alcohols (20.80%), benzenes (5.54%), ketones (4.63%), aldehydes (3.53%), organic acids (2.79%), esters (1.00%), and other minimal compounds (18.08%) (Qian et al., 2006; Hua et al., 2006; Mun and Ku, 2010; Bilehal and Kim, 2012). Of those, phenol homologues and derivates are mainly 2,6-dimethoxyphenol (9.36%), 4-ethylphenol (6.08%), 2-ethylphenol (4.76%), phenol (4.35%), 4-ethyl-2-methoxy-phenol (2.63%) (Qian et al., 2006). The increasing tar accumulation threatens the environment and the sustainability of the charcoal industry, making it necessary to explore effective approaches to exploiting the tar waste or converting it to harmless form for safe release into environment (Qiao et al., 2005; Huang et al., 2013). One of desired approaches is to use bamboo tar as an inexpensive resource for developing botanical insecticides. Recently, two bamboo tar formulations, i.e., emulsifiable concentrate and aqueous solution, were shown to kill leafhoppers, spider mites and aphids (Wang et al., 2015). In this study, we converted the highly viscous tar waste to a water-soluble sodium salt (designated tar salt herein) and evaluated its lethal activity against three global aphid pests in laboratory bioassays and field trials.

1. Preparation of tar salt

The sodium salt was prepared by adding 7.29 g NaOH to 45 g bamboo tar (Zhejiang Tantech Bamboo Technology Co., Ltd., Lishui, Zhejiang, China). The NaOH/tar ratio was determined in repeated

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preliminary tests. The mixture was vigorously stirred using a metal stick until the heat-releasing reaction of bamboo tar with NaOH cooled down. The resulting thin paste, i.e., fully alkalized tar, became completely water-soluble and hence was dissolved in 100 ml of water, forming an aqueous solution of 45% tar salt. The preparation was then used in the bioassays and field trials against the cotton aphid *Aphis gossypii* Glover, the soybean aphid *Aphis glycines* Matsumura and the green peach aphid *Myzus persicae* (Sulzer).

2. Aphidicidal activity of tar salt in laboratory bioassays

The tar salt preparation was diluted by 500, 1000 and 2000 fold with water, yielding the dilutions of 225, 450 and 900 μg tar salt per milliliter. Aliquots of 3 ml each dilution were sprayed on to 9-cm-diameter Petri dishes, each harboring 50 aphids of each species, from the top nozzle of an Automatic Potter Spray Tower (Burkhard Scientific Ltd, Uxbridge, UK) at a working pressure of 0.7 kg/cm^2 (the user's guide). In each dish, five cohorts of 10 laboratory-reared aphids (apterous adults and larger nymphs), which were taken from a laboratory population of each aphid species in professional insectaries, were separately transferred on to 2-cm-diameter leaf discs of cotton (*Gossypium hirsutum* L.) for *A. gossypii*, fava bean (*Vicia faba* L.) for *A. glycines* or cabbage (*Brassica chinensis* L. var. *chinensis*) for *M. persicae*. Approximately 5 ml of sterile water was added to each dish to prevent the leaf discs from desiccation and the aphids from escape. The same-volume spray of water was used as a control. After spraying, all the dishes were covered with lids, maintained for 7 days in a growth chamber at 22–24 °C in a 14:10 light:dark cycle under a relative humidity of 45–55%, and monitored daily for mortality records. Time-concentration-mortality (TCM) responses from the aphid bioassays of three replicates (five cohorts of 10 aphids per capita) were analyzed using a TCM modeling method (Robertson and Preisler, 1992), which enabled the integration of concentration effect, post-spray time effect, and an interaction of both into a single equation (Feng et al., 1998). The fitted parameters for the cumulative effects of concentration and post-spray time and the variances and covariances of both effects were used to compute median lethal concentration (LC_{50}) and associated 95% confidence interval (CI) as a function of post-spray time for the tar salt against each aphid species.

During the period of bioassay, the control mortality of each aphid species was no more than 8%. The sprays of three tar salt dilutions resulted in remarkable TCM responses of each aphid species (Fig. 1). The most concentrated spray caused a corrected mortality of 90% in *A. glycines* and 86% in both *A. gossypii* and *M. persicae* on day 7. The TCM responses of each aphid species fitted the TCM model well ($0.09 \leq P \leq 0.64$ in the Hosmer-Lemeshow C tests for the goodness of fit). Based on the fitted TCM relationships, the LC_{50}s of the tar salt against *A. gossypii*, *A. glycines* and *M. persicae* (Table 1) were estimated as 486, 242 and 489 μg/ml on day 3, respectively. The estimates decreased to121, 88 and 169 μg/ml on day 5, and further to 46, 33 and 103 μg/ml on day 7, respectively. However, these LC_{50} trends were not significantly different among the tested aphid species due to their overlapped 95% CIs (Wheeler et al., 2006). The bioassay results indicate a similar toxicity for the tar salt against the three aphid pests.

3. Aphid control efficacy of tar salt in field trials

Three independent field trials were performed in Zhejiang Province to evaluate control efficacies of the tar salt preparation against *A. gossypii* in a cotton field at Huangbai Village, Yongchang Town, Lanxi County (Trial 1), *A. glycines* in a soybean field at Sanzaofan Village, Yinkeng Town, Kaihua County (Trial 2), and *M. persicae* in a vegetable field at Fanpu Village, Sunduan Town, Shaoxing County (Trial 3). All the located fields were subjected to routine agronomic practices but were not sprayed with any chemical pesticide for up to 45 days prior to the trials. Each field trial comprised five treatments of three or four (for *A. gossypii* only) replicates, which were randomly assigned to 15 or 20 plots (4 × 5 m to 6 × 6 m each) in terms of available field area. A 2-m-wide buffer area was arranged between plots and at the edges of each field. The treatments included three application rates of 45% tar salt solution (168.75, 337.5 and 675 a.i. g/ha), a labeled rate (104.2 a.i. g/ha) of 25% pymetrozine WP (a synthetic aphidicide from Kesheng Inc.,

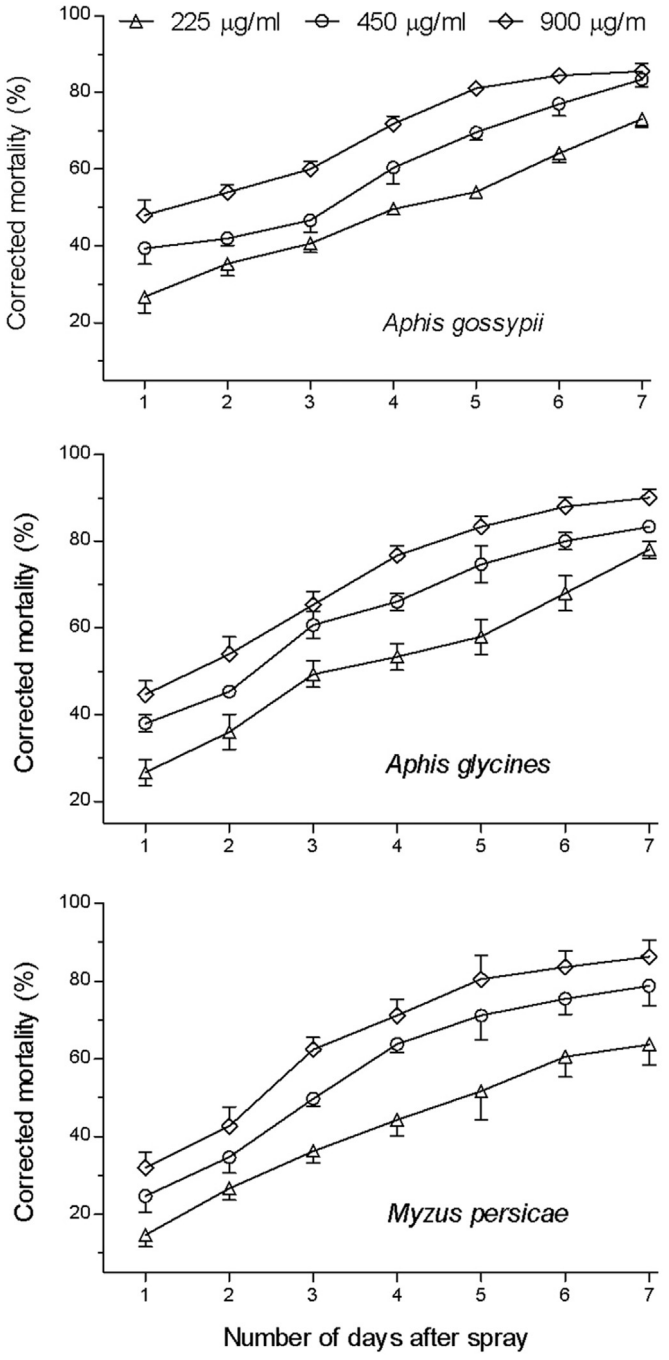

Fig. 1. Corrected mortalities of *A. gossypii*, *A. glycines* and *M. persicae* over the days after aqueous dilutions (225, 450 and 900 μg/ml) of bamboo tar salt were sprayed. Error bars: SE from three replicates of each concentration (50 aphids per replicate).