Industrial Crops and Products 89 (2016) 157–166

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Green tea and its waste attract workers of formicine ants and kill their workers—implications for pest management

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ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 30 March 2016
Accepted 9 May 2016
Available online 19 May 2016

Keywords:
Ants
Tea
Tea waste
Behavioral effects
Toxicity

ABSTRACT

Daily, 3 billion cups of tea consumed worldwide and this consumption is accompanied by the discarding of huge waste amounts into the environment. Tea leaf contains a diverse array of toxic molecules. Despite evidence that its waste is almost as rich in toxicants as green leaves; no research has been done to turn this source of pollution into a benefit for ant pest management, where new chemistries are highly needed as a result on insecticide resistance. The present study was performed to explore the behavioral and lethal effects of tea and its leftovers on the black crazy ant (BCA), *Paratrechina longicornis* Latreille, yellow crazy ant (YCA), *Anoplolepis gracilipes* Smith, and weaver ant (WA), *Oecophylla smaragdina* Fabricius. Both fresh tea extract (FTE) and used tea extract (UTE) were detrimental to the survival of BCA, YCA, and WA. FTE was the most toxic solution and BCA was the most vulnerable species. The presence of tea extracts in meals did not prevent workers of all three species from visiting and feeding in the presence their preferred foods. The results presented here suggest that diets containing tea extracts are attractive to BCA, YCA, and WA when in competition with their preferred foods. These extracts were also insecticidal to the worker ants. These properties demonstrate the potential of tea and its waste products for developing novel environmentally friendly and low-cost ant control strategies, which could also be a practical solution to the growing environmental problem it causes.

1. Introduction

Tea (*Camellia sinensis*; Ericales: Theaceae), which is mainly cultivated in tropical climates (Sarma et al., 2014), is the most widely consumed beverage aside from water in many countries around the world (Primavesi et al., 2014). This consumption is accompanied by the discarding of large amounts of tea waste into the environment (Guardian News, 2014). World tea production and consumption are forecast to significantly increase during the period 2014–2020 (Transparency Market Research, 2015). Tea leaves contain hundreds of chemicals including flavonoids, phenolic compounds, alkaloids and methylxanthines. With the predicted increase in popularity of tea as a beverage, the amounts of tea waste in the environment along with the huge chemical resources that they contain are both expected to increase.

Tea has an array of positive health benefits in humans (Johnson et al., 2012; The Tea Association of the USA Inc., 2013) and negative effects on bacterial and viruses (Heinrich et al., 2011). Tea can also have negative effects on insects. Mitchell et al. (1993) reported increased anti cytochrome P-450-dependent ecdysone 20-monooxygenase activities of flavonoids in dipterans and lepidopterans. Chlorogenic acid has been shown to cause death (Nathanson, 1984), reduce feeding and the bioavailability of amino acids and to decrease digestion in several insects (Dowd and Vega, 1996; Jassbi, 2003; Mallikarjuna et al., 2004).

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http://dx.doi.org/10.1016/j.indcrop.2016.05.019
0926-6690/

In many insects, the ability to develop insecticide resistance is directly influenced by biological and genetic factors (Rust, 1996). This may be the case for any insect pest that has a natural ability to evolve mechanisms to avoid or overcome the toxic effects of chemical agents. Some ants use formic acid produced for attack and defense (Hölldobler and Wilson, 1990), and for detoxifying (LeBrun et al., 2014). Typical species are the yellow crazy ant (YCA) *Anoplolepis gracilipes* (Nelson and Taniguchi, 2012), the black crazy ant (BCA) *Paratrechina longicornis* (LeBrun et al., 2014), and the weaver ant (WA) *Oecophylla smaragdina* (Chung, 1995) (all three Hymenoptera: Formicidae). YCA is regarded as an environmental pest (Gerlach, 2004) and is ranked as one of the world's 100 worst invasive species (Lowe et al., 2000). YCA is widespread in the Asia-Pacific region (Mezger and Pfeiffer, 2011) and is considered the most destructive pest of forest ecosystems (Davis et al., 2010). BCA is an agricultural and household pest in most tropical and subtropical areas (Wetterer, 2008). This species feeds on live and dead insects and many household foods (Smith, 1965). WA is known by its ability to build nests from living leaves (Hölldobler and Wilson, 1990) of many plant species, including fruit trees (Kenne et al., 2003). This ant species is common in oil palm plantations across the Asia-Pacific region (Pierre and Idris, 2013). WA workers hunt and kill insects (Offenberg, 2007).

These Formicidae pests cause significant ecological damage and economic loss. For example, YCA causes notable biodiversity loss in forest ecosystems via its acid spray (Holway et al., 2002). It is also a major threat to electrical wiring of electronic equipment, causing millions of dollars-worth of damage (Coggins, 2013). BCA carries pathogenic bacteria (Fowler et al., 1993) and restricts seed dispersal of myrmecochorous plants (Ness and Bronstein, 2004). WA ants alter the performance and productivity of the host tree by preventing removal of fruit by birds (Thomas 1988) and preying upon insect pollinators (Tsuji et al., 2004).

The main strategies for containing ant pests involve insecticide use through bait (Higgins et al., 1997), residual perimeter sprays (Potter and Hillery, 2002), or both in combination (Vail and Bailey, 2002). Despite some successes, insecticide-based strategies have been impeded by the development of resistance (Ribeiro et al., 2008) and repellent effects (Knight and Rust, 1990). Tea has been proposed to be potentially useful in ant control based on the observation that tea plants are naturally resistant to most insects (The Tea Association of the USA Inc., 2014) and tea oil has been shown to be effective against many bacterial and viral infections (Organic Information Services, 2014) and some insect vectors of disease (Mitchell et al., 1993). As the tea leaf contains a diverse array of toxic molecules with various mechanisms of action (Graham, 1992), it is also likely that tea-based formulations will not trigger the development of resistance. Based on this rationale, we investigated whether fresh and used tea extracts affect the feeding behaviors and survival of crazy and weaver ants.

2. Materials and methods

2.1. Test ant species

Three formicid ant pests—one indoor-dwelling ant species, the black crazy ant (BCA), and two outdoor-living species, the yellow crazy ant (YCA) and the weaver ant (WA)—were used in this study. For all species, two colonies were used as sources of experimental subjects. The BCA colonies were located within a residential buildings, while YCA and WA colonies were in wooded areas within Penang Island, Malaysia. Wild workers of these ant species were regularly obtained from colonies and brought to the insectary of the School of Biological Sciences. (Universiti Sains Malaysia). Workers were kept in aerated plastic bottles with a capacity of 1 L with a bed made from pieces of moist cardboard and a moistened cotton ball to act as a moisture source at the bottom of each bottle. The cotton was rehydrated every 3 days with 10 droplets of distilled water using 3-mL plastic transfer pipettes. The BCA and YCA workers were maintained on 30% sucrose solution. As WA workers were reluctant to feed on the sucrose solution, they were given egg yolk solution prepared from the boiled yolk from one chicken egg smashed in 100 mL of distilled water and sieved through fine mesh mosquito netting. The ants of all colonies were also provided with three fourth-instar mosquito larvae every two days and continuously supplied water though a 1.5-mL Eppendorf tube fixed to the interior bottom edge of each bottle. The maintenance conditions were 29 °C ± 3.0 °C, 75% ± 1% relative humidity (RH), and photoperiod 13:10 h (light:dark) with 1 h of dusk. Colonized workers that had acclimated to laboratory conditions for a minimum of one week were used in the experiments.

Table 1
The experimental extracts used in this study.

Solution	Making procedures
Sucrose solution (30%)	• 30 g of sucrose in 100 mL of deionized water, and allowed to completely dissolve
Egg yolk solution	• One chicken egg was boiled at 100 °C for 10 min; cool full yolk smashed in 100 mL of distilled water; egg solution filtered using a fine mesh mosquito netting
FTE	• 9.4 g of tea leaf in 150 mL of boiled water, and allowed to disintegrate for one hour
FTEs	• Mixture of 15 mL of FTE + 5 mL of a 30% sucrose solution and mix well
SFTE	• Mixture of 5 mL of FTE + 15 mL of a 30% sucrose solution and mix well
UTE	• 9.4 g of used tea leaf in 150 mL of boiled water, and allowed to disintegrate for one hour
UTEs	• Mixture of 15 mL of UTE + 5 mL of a 30% sucrose solution and mix well
SUTE	• Mixture of 5 mL of UTE + 15 mL of a 30% sucrose solution and mix well

2.2. Experimental tea brand and tested experimental solutions

Camellia sinensis var. *sinensis* is an evergreen shrub or small tree. Leaves are used to produce tea drinks. Due to its widely reported health benefits, green tea consumption has increased worldwide (Dufrene, 2013), with accompanying increases in its production (Noguchi-Shinohara et al., 2014). We used Ever Green Chun Mee tea G401/G402, a commercial tea label manufactured by the Shanghai Tiantan International Trading Company, Ltd., China. Chun Mee tea is a kind of green tea grown mainly in Zhejiang province, but also in Jiangxi and Anhui regions (Ministry of Agriculture of China 2010; Vicony Tea Directory, 2014).

The different test solutions were produced according to a modification of the method reported previously (Dieng et al., 2014). Ten replicates of 9.4 g of fresh green tea leaves were transferred into 250-mL plastic containers with 150 mL of boiled water. After 1 h of submersion and dissolution, the tea leaves were removed and placed in new containers labeled accordingly. The 10 tea remnant samples were allowed to air-dry for 1 h. The fresh tea infusions were sieved through fine mesh mosquito netting and the resulting solutions were designated as fresh tea extracts (FTE). A volume of 150 mL of boiled water was added to each of the 10 contain-