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Toxicity and repellent action of *Coffea arabica* against *Tribolium castaneum* (Herbst) adults under laboratory conditions

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ABSTRACT

Coffea arabica parchment extracts and caffeine isolated from the plant were evaluated randomly against 1-week-old adults of red flour beetle, *Tribolium castaneum* for fumigant toxicity and repellent action under laboratory conditions. The effects on detoxification enzymes and neuroenzyme was also determined. Among the various extracts prepared sequentially using hexane, dichloromethane, ethyl acetate and methanol as solvents, the dichloromethane extract did repel insects on contact (EC_{50} = 4380.65 ppm). The repellence was more prominent when an olfactory response was measured (EC_{50} = 2571 ppm). The active ingredient isolated from dichloromethane extract was identified as caffeine which showed very strong repellency as compared to the extract. In terms of toxicity of this extract, a significant mortality was recorded in fumigation assay (LC_{50} = 5555 and 791 ppm, 24 and 48 h post-treatment, respectively). However, caffeine did not induce similar toxicity as the dichloromethane extract. The studies on the impact on detoxification enzymes of *T. castaneum* showed that dichloromethane extract inhibited carboxyl esterase activity, which possibly led to high toxicity. However, caffeine inhibited glutathione-s-transferase and induced carboxylesterase enzymes. It was, therefore, obvious that *C. arabica* parchment crude extracts have dual effects against *T. castaneum* adults, *i,e*, fumigant toxicity and repellent effects. However, the active compounds responsible for the two activities are surely different as caffeine could only induce repellent action against the beetles and the toxic compound needs to be identified, which is presently being investigated.

1. Introduction

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is an economically important stored grain insect pest in the tropical and temperate regions which feed generally on wheat flour and other food products including, cereals, pasta, biscuits, beans, and nuts (LeCato, 1975; Hameed and Khattak, 1985; Irshad and Talpur, 1993; Suresh and White, 2001; Hagstrum et al., 2012) causing severe damages to quantity and quality of these food items (Smith et al., 1971). These infestations have serious impact on the global economy (Domínguez and Marrero, 2010) and annual overall damage has been reported as 10–40% of total worldwide products (Farzana et al., 2013). In addition, members of genus *Tribolium* are reported to secrete certain toxic quinones of carcinogenic nature in stored commodities thus posing serious risks to human health (Ladisch et al., 1967; El-Mofty et al., 1989; Domínguez and Marrero, 2010). The general approach to control this pest for decades has been the use of chemical fumigants, which have several deleterious effects such as their persistent toxicity in food grains, the subsequent development of resistance in insect populations, effects on non-target organisms and toxic to users (Champ and Dyte, 1976; Mohan et al., 2010). Thus, the search for eco-friendly approaches is essential non-chemical methods (Flinn and Hagstum, 2001), biological control (Arbogast, 1984; Guedes, 1990; Brower et al.,

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1996) or safer biopesticides like plant based toxins (Chiasson et al., 2004; Koul, 2016).

Use of plant materials with insecticidal properties against stored grain pests has potential in terms of local availability, biodegradable characteristics (Golob et al., 1999), and inexpensive methods for control (Mishra et al., 2012). The main advantage of botanicals is that they could be prepared easily by farmers, small-scale industries and are potentially less expensive (Nikkon et al., 2009). Most of the plants have developed defence mechanisms against natural enemies in the course of evolution. Among these are morphological and chemical defense mechanisms against insects and other parasites that do not generally cause immediate death but interfere with their vital biochemical and physiological functions (Prakash and Rao, 1997). Several local species have been reported as repellents and toxic to red flour beetle, *T. castaneum*. For example, *Ambrosia tenuifolia* Spreng. (Asteraceae), *Baccharis trimera* (Less.) (Asteraceae), *Brassica campestris* L. (Brassicaceae), *Jacaranda mimosifolia* D. Don (Bignoniaceae), *Matricaria chamomilla* L. (Asteraceae), *Schinus molle* (L.) var. areira (L.) DC (Anacardeaceae), *Solanum sisymbriifolium* Lam. (Solanaceae), *Tagetes minuta* L. (Asteraceae) and *Viola arvensis* Murray (Violaceae) are insecticidal to coleopteran pests of stored grain including *T. castaneum* (Padín et al., 2000; Tsao et al., 2002; Al-Jabr, 2006; Juan Hikawczuk et al., 2008; Benzi et al., 2009; Arora et al., 2011). Similarly, there are reports to show that *Coffea arabica (*Rubiaceae) has repellent, insecticidal, antifeedant, and growth regulatory properties against various insect pests like *Leptinotarsa decemlineata*, *Streptomyces scabies* and *Clavibacter michiganensis* (Rohan et al., 2010); *Aedes aegypti* (Laranja et al., 2003) and *Ochlerotatus notoscriptus* larvae (Derraik and Slaney, 2005). The major compound of coffee bean has already been identified as caffeine (1, 3, 7- trimethylxanthine) which is a member of purine alkaloids and one of the most widely used plant secondary metabolite (Sano et al., 2010). However, there is no record to show the effect of parchment part of coffee bean on stored pests. Therefore, the present study was designed to investigate toxic action of parchment part extracts of *Coffea arabica* against adults of *T. castaneum*. Another reason to use parchment part of coffee berries was that in coffee industry the parchment is always removed and is generally a waste (Selmar and Knopp, 2008), therefore, the idea was to use this waste in order to utilize such unwanted raw materials for the development of the biopesticide products that could be economically valuable replacement for conventional pesticides at reasonably cheaper rates.

2. Materials and methods

2.1. Insect

The red flour beetles, *T. castaneum* were obtained from Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand and were maintained in Animal Toxicology and Physiology Speciality Research Unit as laboratory cultures in the dark condition in incubators at 27–28 °C and 70–80% RH. *T. castaneum* were reared on wheat flour mixed with yeast (10:1, w/w). Unsexed adult beetles (*ca.* 1 week old) were used in all the experiments.

2.2. Plant materials

Dried *Coffea arabica* L. cv. Catimor parchments (10 Kg) were collected from Chiangrai province, Thailand. The pericarp was ground to a powder that was subsequently extracted by soaking in hexane, dichloromethane, ethyl acetate and methanol, respectively. Each extract was filtered using a vacuum pump and dried using a rotary evaporator (BUCHI R-215, Japan) and stored at 4 °C until further use in the experiment. The percentage yield was calculated using the formula: Yield (%) = $W/W_0 \times 100$, where W is the weight of dried extract and W_0 is the weight of the sample (Santos et al., 2012).

2.3. Caffeine

The most active dichloromethane extract was subjected to preparative thin layer chromatography (Silica gel 60 PF254, Merck) and eluted with ethyl acetate to afford fraction 1 and 2. Fraction 2 was further purified by preparative thin layer chromatography using 100% hexane and then 100% ethyl acetate to obtain caffeine that was confirmed by spectral analyses.

2.4. Repellent assay by filter paper method

A completely randomized design method was chosen to evaluate the repellency. Each extract and caffeine was diluted with acetone solutions (AR grade). One half of a filter paper (Whatman No.1, diameter 9.0 cm) was treated with 0.5 ml of the relevant substance while the other half was treated with 0.5 ml acetone as a control and allowed to air dry for 3 min. Then the halves were re-attached on bottom using adhesive tape, and kept in 9-cm glass Petri dishes. One week old *T. castaneum* adults were released at the center of each filter paper disk (CRD with 10 adults/dose, 3 replicates). The dishes were covered and placed in darkness at 26 °C and relative humidity of 65 ± 5%. The numbers of *T. castaneum* adults on the treated and untreated portions of the experimental paper halves were counted for each dish after 2 h exposure. The tests were repeated from 13:00 h to 19:00 h. The EC_{50} was calculated using Probit analysis by the StatPlus 2008 software package. Differences between results were determined using Tukey in the SAS program.

2.5. Repellent assay by olfactometer method

A Y-tube olfactometer was used for investigating a preference of individual insects exposed to a binary choice of odours. This olfactometer was made of glass and consisted of a central tube (L = 25 cm, ∅ = 3.5 cm) connected to two arms (L = 22 cm, ∅ = 3.5 cm). Compressed air flowed through both arms, thus creating an air stream of 144 ml/min per arm. A light source was positioned in such a way that each arm received equal amount of light. The temperature was maintained at 26 °C at all times. After filtering with activated charcoal and moisturing, the air was passed through two glass odour flasks. The first flask had the filter paper (3 × 3 cm) containing 0.5 mL each of crude extract or caffeine diluted in acetone. Second flask represented the controls and contaoned 0.5 mL acetone (AR grade) only. Before filter paper was placed in a flask, it was allowed to dry at room temperature for 10 min. One randomly selected adult was placed individually at the entrance of the central tube and allowed to choose between the two arms. The tests were repeated from 13:00 h to 19:00 h. The experiments were repeated 30 times for each concentration. The EC_{50} was calculated using Probit analysis by the StatPlus 2008 software package. Differences between results were determined using Tukey in the SAS program.

2.6. Fumigant and contact toxicity bioassay

The fumigant toxicity was conducted by using impregnated filter paper method with Completely Randomized Design in 10 replicates. The filter papers (Whatman No.1) was cut into 3 cm diameter pieces and impregnated with 3 μl extract diluted with acetone (AR Grade) to give final dose between 0 and 20,000 ppm. In