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Survival of Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) in apples treated with essential oils and cold storage

Yusup Hidayat[a,b,*], Neil Heather[a], Errol Hassan[a]

[a] School of Agriculture and Food Sciences, The University of Queensland, Gatton, QLD 4343, Australia
[b] Department of Plant Pests and Diseases, Padjadjaran University, Sumedang, West Java 45363, Indonesia

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ABSTRACT

Little is known on the fumigant effects of essential oils on the immature stages of fruit flies (eggs and larvae). The present study aimed to investigate effects of fumigation with essential oil alone or followed by cold storage on the survival of Queensland fruit fly *Bactrocera tryoni* in Gala apples. Efficacy was based on the number of pupae recovered from treated and untreated fruits and on phytotoxic effects. In a 24 h fruit fumigation test, peppermint oil applied at 100 and 200 μL/L air was found to be active against *B. tryoni* eggs, whereas broad-leaved peppermint oil was active against both eggs and larvae but only at the highest dose tested (200 μL/L air). However, both peppermint and broad-leaved peppermint oils sometimes had a phytotoxic effect on the apples. In a 6 h fruit fumigation test, an equal mixture of peppermint and broad-leaved peppermint oil (100 μL/L air) did not cause phytotoxic effect but had only a slight effect on *B. tryoni* eggs and no effect on the larvae. There was no synergism or additive effect when this essential oil mixture was applied in combination with subsequent cold storage. These results indicate that peppermint oil and broad-leaved peppermint oil have little potency for Gala apple fumigation since they were only effective at doses and durations of exposure which were phytotoxic to fruit. On the other hand, cold storage (4 ± 1 °C) alone was confirmed to be a very effective treatment against *B. tryoni* larvae and eggs in Gala apple without causing fruit damage and was not enhanced by prior fumigation with these oils.

1. Introduction

Plant essential oils are among natural products that have potential to be developed as fumigants. They have been reported to have fumigant effects on a number of postharvest as well as preharvest insect pests (Yi et al., 2006; Rajendran and Sriranjini, 2008; Kimbaris et al., 2010). In direct exposure (in vitro) testing of eight potentially active essential oils, peppermint (*Mentha piperita*) was the most efficacious on eggs whereas broad-leaved peppermint (*Eucalyptus dives*) was the most efficacious on larvae of Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Hidayat et al., 2013). However, the fumigant effect of an essential oil on fruit fly eggs and larvae in the infested fruits is apparently untested.

Peppermint, *M. piperita* is a herb plant belonging to the family Labiatae (Small, 2006). Its essential oil, which is rich in menthol, is a popular flavouring agent for foods such as sweets and chewing gum and for oral health care products such as toothpaste and mouthwashes. This essential oil is also used in alternative medicines (Keifer et al., 2007). Broad-leaved peppermint, *E. dives* is a medium sized tree (Boland et al., 2006) with a strong peppermint odour from the crushed leaves (Brooker and Kleinig, 2006). The essential oil of this Australian native plant contains large amount of piperitone (Bignell et al., 1998; Delaquis et al., 2002; Weber et al., 2006; Gilles et al., 2010), which is a raw material in the production of synthetic menthol (Leffingwell and Shackelford, 1974). Not much is known of the medicinal properties of this essential oil, but there have been reports on its antimicrobial activities (Delaquis et al., 2002; Gilles et al., 2010).

Methyl bromide is currently the most widely used fumigant for phytosanitary purposes (Heather and Hallman, 2008) including disinfestation of tephritid fruit flies in fresh fruits (Armstrong, 1992; Hallman and Thomas, 2011; Dominiak and Ekman, 2013). Methyl bromide is known to have strong penetrative ability and to be tolerated by many commodities (Follett and Neven, 2006). However, under the Montreal protocol due to its ozone depleting effects, the production and usage of methyl bromide has been scheduled to be phased out in developed countries (with few exemptions) by 1 January 2005 and by 1 January 2015 in

* Corresponding author at: Department of Plant Pests and Diseases, Padjadjaran University, Sumedang, West Java 45363, Indonesia. Fax: +62 22796316.
E-mail address: ysphdyt@yahoo.com (Y. Hidayat).

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developing countries (UNEP, 2009). Although quarantine and pre-shipment uses of methyl bromide are exempted from the phase out (UNEP, 2009), countries have been urged to replace or reduce the use of methyl bromide in phytosanitary measure if alternatives are technically and economically feasible (IPPC, 2008). This recommendation is considered to be necessary to further reduce reliance on methyl bromide.

Ideally, methyl bromide alternatives for postharvest treatment need to be as effective as methyl bromide, active against all life stages of insects, safe to the environment (and definitely to humans), able to prevent target insects from becoming resistant to treatments, safe for the products (no phytotoxic effects), short action times, low cost and be easy to apply (Aegerter and Folwell, 2000). However, it seems that there are no alternatives that fully meet the above criteria. For example, phosphine is widely used for grain fumigation due to its effectiveness, is simple to apply, lacks significant residue and has low cost (Nath et al., 2011), but this toxic chemical has slow action (several days or longer) (Chaudhry, 1997) so that it may not be suitable for perishable products such as for many fruits and vegetables. There is also a growing concern on the development of phosphine resistance in a number of stored product insects (Opit et al., 2012; Nayak et al., 2013). Another alternative, irradiation, may be available for disinfestation of many fruits but it is not a cheap treatment (Aegerter and Folwell, 2000). Cold storage is a simple and safe treatment, but it is only suitable for fruits that can tolerate long exposure to low temperatures (Armstrong, 1992; Aegerter and Folwell, 2000; Heather and Hallman, 2008). Therefore, it seems the most realistic alternatives for postharvest treatments are combinations of treatments.

Nevertheless, exploration of new chemicals for fruits fumigation should continue in order to identify those with strong fumigant effects against arthropod pests but be safe to humans and the environment. The current study aimed to investigate: (a) effects of fruit fumigation with peppermint and broad-leaved peppermint oils on the survival of *B. tryoni* in the treated fruit and (b) effects of fruit fumigation with peppermint and broad-leaved peppermint oil mixture followed by cold storage on the survival of *B. tryoni* in the treated fruit.

2. Materials and methods

2.1. Plant essential oils

Peppermint (*M. piperita*) and broad-leaved peppermint (*E. dives*) oil were supplied by BioAust Pty Ltd. (Jimboomba, QLD, Australia) and refrigerated prior to use.

2.2. Insects

Pupae of *B. tryoni* were obtained from the (Queensland) Department of Employment, Economic Development and Innovation (DEEDI), Indooroopilly then reared as an ongoing experimental population at the University of Queensland, Gatton Campus. The general rearing technique for *B. tryoni* was as described by Heather and Corcoran (1985). Adults were supplied with sugar cubes, yeast hydrolysate (MP Biomedicals) and water and maintained at 25 ± 1 °C, RH 60 ± 10%, and 14:10 (light:dark) photoperiod. Adults laid eggs in an artificial fruit made from a 500 mL glass jar filled with 30 mL apple juice and covered with plastic film tightened with a rubber band. Fruit fly eggs were collected, washed with water, filtered with a fine mesh and transferred onto filter paper (9 cm diameter, Whatman No. 1). One-day-old eggs were placed on larval diet in a plastic container. The larval diet consisted of dehydrated carrot (75 g), torula yeast (25 g), nipagin (2.5 g), hydrochloric acid (3 mL) and water (750 mL). For pupation, a 1 cm layer of sand was provided. Pupae were collected by sieving the sand and then held in a plastic container (1 L) filled with moistened vermiculate for adult emergence. At one day prior to a trial, mature female flies were trapped from cages using a modified plastic container (1 L) with apple juice (applied to a rolled paper towel) as an attractant. After that, they were immobilized in a cold room (7–8 °C) and immediately placed in 1 L plastic containers (5 female flies per container). The containers were covered with mesh tightened with rubber band and then transferred into a room maintained at 25 ± 1 °C and 14:10 (light:dark). The flies were fed with yeast hydrolysate and sugar cubes. Water was sprayed onto the container walls.

2.3. Twenty-four hour fruit fumigation with peppermint and broad-leaved peppermint oils

Peppermint and broad-leaved peppermint oil at three dose levels (50, 100 and 200 μL/L air) were evaluated for their fumigant effects on *B. tryoni* eggs and larvae in apple fruit. Prior to experimentation, Gala apples (organic) were washed and prepunctured five times with an entomological needle (1 mm deep). Each apple was then placed in a plastic container (1 L) containing five mated female *B. tryoni* (14–16 days old). After one hour of exposure to females, apples were removed from the plastic containers and kept at 25 ± 1 °C. Two experiments were conducted, first against the egg stage and then against the larval stage. In the first experiment, apples were fumigated with the essential oils at one day after oviposition. Apple fumigation used filter paper method with a 950 mL glass jar as the fumigation chamber. This fumigation method was adapted from Germinara et al. (2007). First, an apple was placed inside a glass jar and the required amount of each essential oil was pipetted on a 5 cm × 7 cm filter paper (Whatman No. 1) suspended from the underside of the glass jar lid. For control, no essential oil was applied. The glass jar was then closed tightly with a glass stopper fitted to a hook. After 24 h, fumigation was terminated and the treated apples were aerated under laminar flow for 15 min. Each apple was then placed in a 1 L plastic container lined with two layers of paper towel and covered with fine mesh (tightened with rubber band). Eight days after fumigation, apples were cut and live larvae were transferred into 30 mL plastic containers containing larval diet. The number of normal pupae from fumigated and control fruits was observed and recorded. Experiment was held in a randomized complete block design with four replications. In the second experiment, apples were fumigated with the essential oils at four days after oviposition using the same fumigation method.

2.4. Six hour fruit fumigation with an essential oil mixture followed by cold storage

Effects of six hour fruit fumigation with an essential oil mixture in combination with cold storage on the survival of *B. tryoni* in apples were investigated. Two separate experiments were conducted against egg and larval stage. Both experiments used a factorial design. The experiment against the egg stage was done at 1 day after oviposition using a 2 × 3 factorial design consisting of essential oil mixture factor (0 and 100 μL/L air) and cold treatment factor (0, 5 and 6 days). Against the larval stage, the experiment was done at 6 days after oviposition using a 2 × 4 factorial design consisting of essential oil mixture factor (0 and 100 μL/L air) and cold storage factor (0, 6, 7 and 8 days). The essential oil tested was an equal mixture of peppermint oil and broad-leaved peppermint oil (v/v). Fruit fumigation was done using a filter paper method with a 950 mL glass jar as the fumigation chamber. Following fumigation, apples were removed from fumigation chamber,