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INSECTICIDAL ACTIVITY OF ESSENTIAL OIL FROM SEEDS OF *PONCIRUS TRIFOLIATA* (L.) RAF.

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Abstract

The essential oil from the seeds of oriental medicinal plant *Poncirus trifoliata* (L.) Raf. was tested for repellent and fumigant efficacy on fruit fly (*Drosophila melanogaster*) and mosquito (*Anopheles culicifacies*). The oil showed potent activity with 76.2 and 93.2% mortality in fumigant assay, and 77.1 and 93.2% repellent efficacy on fruit fly at doses of 100 and 200 μg/ml, respectively. The application of oil against mosquito showed 80.0% mortality in fumigant assay and 70.3% repellency at 100 μg/ml dose. Thus, essential oil of *P. trifoliata* seeds can be considered as a potential source of biologically active compounds for pest control.

Introduction

The fruit fly, *Drosophila melanogaster* belonging to the family Drosophilidae (Order: *Diptera*) is well-known as a nuisance pest, and very often, they could be harmful to human, animals and fruits. Generally, fruit flies are found in the area of rotten garbage, human or animal waste and carry germs from those areas, and contaminate foods and household utensils. Actually, the flies vomit and leave excrement during their roaming on an object and thus they spread diseases to cause food poisoning and dysentery. Again, adult fruit flies most often lay their eggs in the fresh flesh of fruits and vegetables and the larvae (maggots) hatched from those eggs are feed from those fruit or vegetables, resulting in soft, mushy mess i.e. damaging fruits. Recently, mosquito species are considered as very serious threat to human health worldwide as mosquito plays very important role in transmitting major human diseases and thus imposing a significant economic burden especially to the countries of tropical regions (Karimian *et al.* 2014). As per report of World Health Organization (WHO 2013), mosquitoes are responsible for transmitting diseases to more than 700 million people and 655,000 deaths from malaria every year. *Anopheles culicifacies* is one of the primary malarial vectors (Surendran *et al.* 2006).

Attempt to control pest and manage harm caused by insects has a long and varied history. Synthetic insecticides and fumigants are extensively used for pest control. For instance, bendiocarb (carbamate), malathion (organophosphate), deltamethrin, cyfluthrin, α-cypermethrin and lambdacyhalothrin (synthetic pyrethroids) are widely used as insecticides nowadays (Amerasinghe *et al.* 1999). Unfortunately, *A. culicifacies* has already been found to develop tolerance against all those evaluated insecticides (Raghavendra *et al.* 2014). Moreover, these synthetic insecticides have been reported to cause numerous problems such as disturbances of the environment, increasing costs of application, pest resurgence, resistance to pesticides, and lethal effects on non-target organisms in addition to direct toxicity to the users (Wang *et al.* 2011).

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In recent years, the essential oil possessing insecticidal properties has attracted great attention in pesticide research (Stefanazzi *et al.* 2011). Botanical pesticides have multifold advantages, i.e. they can provide novel modes of action against insects, may reduce the risk of cross-resistance and offer new leads for designing specific target based molecules (Li *et al.* 2011). Essential oils (EO) are complex mixture of volatile molecules, and have been widely investigated for their efficacy as fumigant, and repellant agent, and found quite promising in developing natural repellents or insecticides (Dutra *et al.* 2016).

Poncirus trifoliata (L.) Raf. (Rutaceae) known as trifoliate orange or Korean bitter orange is a deciduous or semi-deciduous shrub, native to China and Korea. Traditionally, trifoliate orange (*P. trifoliata*) has been widely used in folk medicine as a remedy for gastritis, dysentery, inflammation and digestive ulcers (Yeung 1985). *P. trifoliata* fruit derived compounds have been reported to have various biological activities including anti-inflammatory, antibacterial and anti-anaphylactic (Kim *et al.* 1999), apoptosis of cancer cells (Rahman *et al.* 2015) and antilisterial (Rahman *et al.* 2012) properties. Several compounds such as poncirin, coumarins, auraptine, hesperidin and naringin have been identified from poncirus fruits (Avula *et al.* 2005). Previously, investigation on chemical composition of the seed essential oil by GC-MS method, and the antibacterial potential of the oil and organic seed extracts of *P. trifoliata* against foodborne pathogens had been reported (Rahman *et al.* 2009). However, so far, there is no available report on insecticidal i.e. fumigant and repellent activity of seed essential oil of *P. trifoliata*. The aim of this study was to determine the repellent and fumigant properties of essential oil against fruit fly (*Drosophila melanogaster*) and mosquito (*Anopheles culicifacies)* to provide an insight about the potentiality of essential oil as effective alternatives to synthetic insecticides.

Materials and Methods

The fruits of *Poncirus trifoliata* were collected from the local area of Kyoungsan, Republic of Korea in September and October, 2007. The plant was identified at the Department of Biotechnology, and a voucher specimen was deposited in the herbarium of College of Engineering, Department of Biotechnology, Daegu University, Republic of Korea.

The seeds were separated from fruits, washed with distilled water and air-dried at room temperature for 5 days. The dried seeds were then pulverized into powdered form by a grinding machine. The dried seed powder (200 g) of *P. trifoliata* was then subjected to hydro-distillation using a Clevenger-type apparatus for 3 hrs. The hydrodistillation of the seeds of *P. trifoliata* gave a yellowish oil with a yield of 0.34% (w/w). The volatile oil was dried over anhydrous Na_2SO_4 and preserved in a sealed vial at 4^{o}C until further analysis.

To assess the fumigant activity against fruit fly (*Drosophila melanogaster*), the method described by Karr and Coats (1988) was followed. In this study, closed plastic bottles of dimensions (30 × 15 cm), capped with polypropyle stoppers were used. Each of the oranges used in this experiment was cut into two pieces and then the orange pieces were treated with 1 ml solution of oil of 1, 10, 100 and 200 μg/ml (5% DMSO was used in dissolving oil). Twenty adult fruit flies were released into each bottles under experiment. The control was tested applying only 5% DMSO. Then bottles were covered with fine nylon cloth using adhesive tape. The bottles were maintained at 30 ± 1°C with a photoperoid of 16 hrs light/8 hrs dark cycles. Each of the experiment was replicated thrice. The mortality was assessed after 6, 12, 24, 36, 48 and 72 hrs of treatment.

For repellency assay, the method described by Talukder and Howse (1995) was followed. Each of the orange slices (each of the orange was cut into two halves) was treated with any of the specified doses (1, 10, 100 and 200 μg/ml) of seed essential oil, and placed in a plastic bottle (30 ×

15 cm), which was then connected with the help of nylon cloth and adhesive tape to the control bottle containing other half of the orange treated with only 5% DMSO. Twenty adult flies were released in these bottles. Only 5% DMSO was applied for control group. The bottles were maintained at 30 ± 1°C with a photoperoid of 16 hrs light/8 hrs dark cycles. The percentage of repellency was calculated after 6, 12, 24, 36, 48 and 72 hrs of treatment.

For fumigation assay against mosquitoes, the method developed by Rice and Coats (1994) was followed but with slight modification. This assay was carried out using 25, 50 and 100 µg/ml essential oil using plastic bottles of dimension of 12 × 12 cm capped with polypropyle stopper. A group of 10 adult mosquitoes (*Anopheles culicifacies*) were transferred to a bottle and then the bottle was connected with another bottle containing specified amount of essential oil. The bottles were covered with fine nylon cloth using adhesive tape. The bottles were then turned upside down in such a way to keep the bottle containing seed essential oil below, which aided to saturate the atmosphere of the bottle containing mosquitoes by essential oil vapor. The control consisted of a similar setup with only 5% DMSO. The bottles were maintained at room temperature and mortality was counted after 1, 2, 3, 6, 9 and 12 hrs of treatment.

The arrangement for the repellency test against mosquitoes was similar to the arrangement against fruit fly. In this case, essential oil was tested for the concentrations of 25, 50 and 100 µg/ml. The control group treated with only 5% DMSO. Ten adult mosquitoes were used in a group and all the tests were replicated thrice. Repellency percentage was counted after 1, 2, 3, 6, 9 and 12 hrs of treatment.

Data were analyzed by means of ANOVA test followed by t-test. Significant levels were determined at $p < 0.05$ (95% confidence limit).

Results and Discussion

Fumigant effect of seed essential oil on fruit fly: The results of the toxic effect of seed essential oil are presented in Fig. 1. The seed essential oil showed its efficacy against fruit flies up to 90% in a dose- and time-dependent manner. At the doses of 100 and 200 µg/ml, the seed essential oil revealed the mortality of 76.2 and 93.2%, respectively after 36 hrs exposure and this rate of mortality was found same up to 72 hrs. However, for lower dose of oil (1 µg/ml), no significant toxic effect on fruit flies was found.

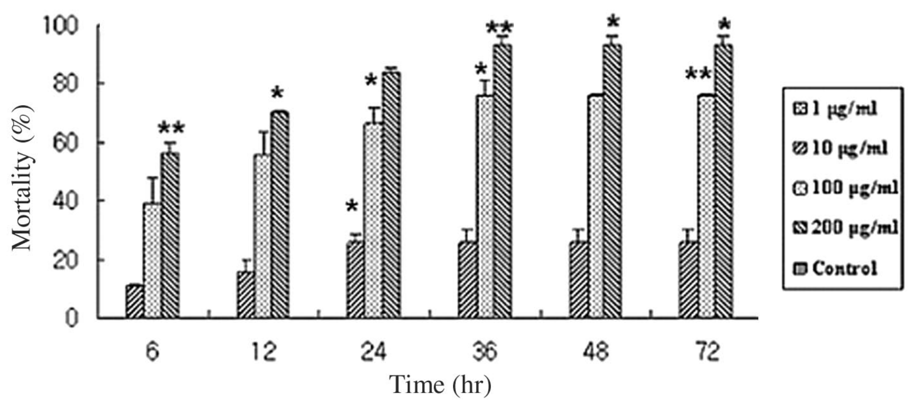

Fig. 1. Insecticidal effect of seed essential oil of *Poncirus trifoliata* on viability of fruit fly (*Drosophila melanogaster*). Control: 5% DMSO. ($p < 0.05$ and $p < 0.01$ compared with control group).

Repellent effect of seed essential oil on fruit fly: The seed essential oil showed a strong repellency to adult fruit flies and the percentage of repellency is presented in Fig. 2. At the concentration of 100 and 200 µg/ml, the seed essential oil exhibited 77.1 and 93.2% repellency, respectively after 72 hrs of treatment. No effect was found for DMSO in control group. The repellency effect of seed essential oil was found to increase with the increase of time and concentrations indicating the repellent effect was time and dose dependent.

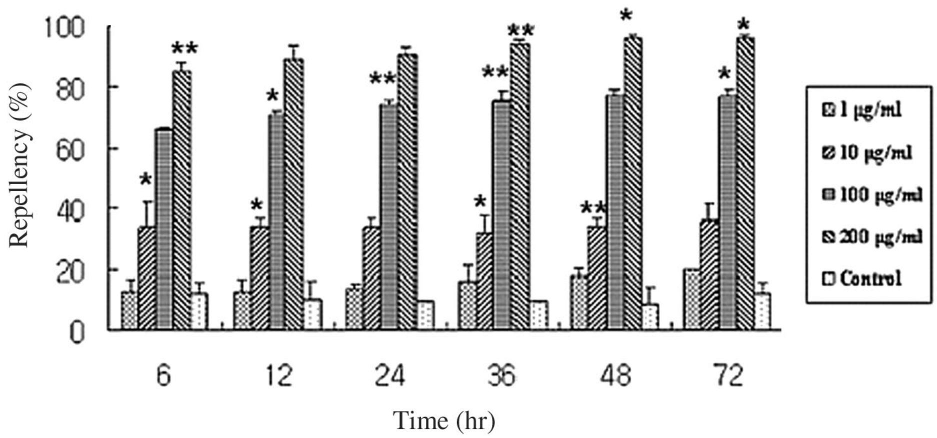

Fig. 2. Repellent effect of seed essential oil of *Poncirus trifoliata* on viability of fruit fly (*Drosophila melanogaster*). Control: 5% DMSO. ($p < 0.05$ and $p < 0.01$ compared with control group).

Fumigant effect of seed essential oil on mosquito: The toxic effect of seed essential oil on mosquitoes is presented in Fig. 3. The oil showed potent fumigant activity on adult mosquitoes in a dose dependent manner. The mortality rate was 25.1, 50.2 and 80.0% at the doses of 25, 50 and 100 µg/ml, respectively after 6 hrs of treatment and these rates of mortality were found up to 12 hrs. However, no effect was found for the control (5% DMSO) used in the experiment.

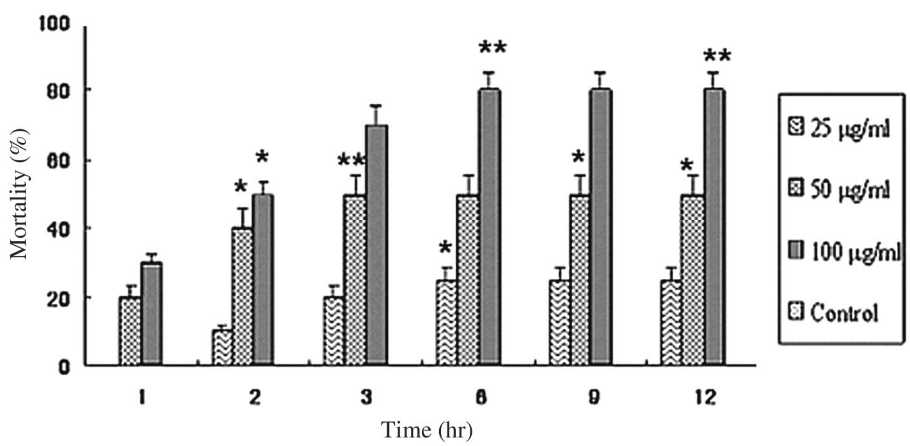

Fig. 3. Insecticidal effect of seed essential oil of *Poncirus trifoliata* on viability of mosquito (*Anopheles culicifacies*). Control: 5% DMSO. ($p < 0.05$ and $p < 0.01$ compared with control group).

Repellent effect of seed essential oil on mosquito: Different doses of seed essential oil demonstrated significant dose-dependent repellency effect on adult mosquitoes as presented in Fig. 4. With the increase of oil concentration from 25 to 100 μg/ml, the repellency rate also increased from 20.2 to 70.3% as compared to the control group. There was no observable effect for DMSO treatment. It is also apparent that the repellent effect exerted by the oil was significantly influenced by the doses applied and the duration of exposure (Fig. 4).

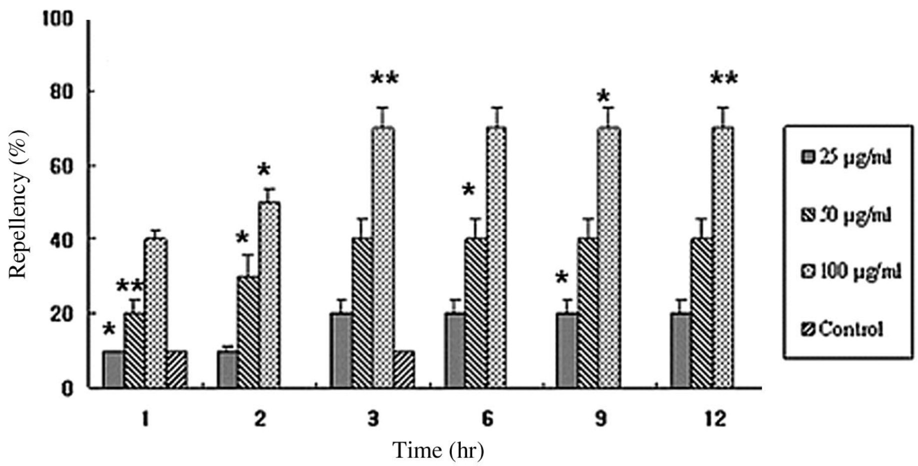

Fig. 4. Repellent effect of seed essential oil of *Poncirus trifoliata* on viability of mosquito (*Anopheles culicifacies*). Control: 5% DMSO. ($p < 0.05$ and $p < 0.01$ compared with control group).

In pest control, fumigant compounds are generally used when it becomes necessary to destroy the insect populations directly, whereas, the repellents are more appropriate when the aim of the pest control is not to destroy the population of certain insect species but to reduce their density in specific area of crops or fruits. In this study, the seed essential oil of *P. trifoliata* showed both of the fumigant toxicity and repellent effect significantly against fruit fly and mosquito. In some other studies, different essential oils were found to have similar activities (Konstantopoulou *et al*. 1992, Jacobson 1989) and the results of the present study are in good agreement with those reports. It has been reported that the monoterpenes, sesquiterpenes and oxygenated sesquiterpenes present in essential oils are mainly responsible for insecticidal activity of oil (Isman 2000). The essential oil of *P. trifoliata* was found to contain a complex mixture of sesquiterpenes and oxygenated sesquiterpenes, where veridiflorol (17.34%), spathulenol (14.21%) and α-caryophyllene (12.32%) were identified as major components of the oil (Rahman *et al.* 2009). The insecticidal properties of veridiflorol, spathulenol and caryophyllene against various insects have already been well documented (Jaenson *et al.* 2006, deMorais *et al.* 2007). In the present experiments, veridiflorol, spathulenol and caryophyllene might play the central role behind the insecticidal effect of the essential oil. The presence of other minor components may contribute some synergetic effect in the insecticidal potential of the oil. Furthermore, as essential oils are comprised of broad-spectrum of components, their mode of action in exerting insecticidal effect could be different (Chiasson *et al.* 2004). As the essential oil under investigation was rich in sesquiterpenes and oxygenated sesquiterpenes, the insecticidal activities might be due to the effect on octopaminergic nervous system of fruit flies or mosquitoes (Enan 2001).

Controlling harmful insects is a global challenge that is growing at an alarming pace these days, more particularly in the developing countries. In order to diminish harmful effects produced by insects, the use of biologically active compounds from natural products needs to be explored. Moreover, the search for novel natural insecticides of plant origin has also been increased. The seed essential oil of *P. trifoliata* would seem to be useful as a natural insecticidal since it contains biologically active vapors of potential secondary metabolites to serve the purpose. This study may also open the window to explore this plant as source of active compounds for bio-preservatives and agro-based pharmaceutical industries.

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