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Screening of Juvenile Hormone Disruptors from *Myzus persicae* using Yeast β-galactosidase Assay

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Abstract This study established a basis for the development of eco-friendly pest control agents using plant extracts that interfere with the binding of insect juvenile hormones. Owing to its substantial influence on crop production, the peach aphid (*Myzus persicae*) is a known economic pest. To produce transformants for the screening of plant extracts, a yeast two-hybrid assay was performed with the juvenile hormone receptor Methoprene-tolerant of *Myzus persicae* and its partner protein steroid receptor co-activator. Members of the Cupressaceae, Pinaceae, Theaceae, and Magnoliaceae families were identified as high-ranking plant groups based on the results of the screening for plant extracts with elevated juvenile hormone disruptor (JHD) activity. High JHD activity was confirmed in extracts of various species, including *Camellia japonica*, *Lindera erythrocarpa*, *Carpesium abrotanoides*, *Magnolia obovata*, and *Hedera rhombea*. Based on these results, the extracts that showed considerable JHD activity are considered safer and more environmentally friendly than existing pest control agents and, hence, could be used as a basis for the development of new pest control agents.

Key words Juvenile hormone, Juvenile hormone disruptor, Methoprene-tolerant, Plant extracts, Steroid receptor co-activator

Introduction

Peach aphids, *Myzus persicae* (Sulzer 1776; Hemiptera: Aphididae), mediate and transmit over 100 types of plant viruses (Blackman and Eastop, 2000), causing damage to both horticultural plants and economically important crops. They reduce the value of agricultural products by interfering with plant growth (Powell et al., 2005; Goggin et al., 2017), and they are one of the most economically important pests in the world (van Emden and Harrington, 2007). Although various methods have already been adopted for the control of peach aphids, the demand for environmentally friendly pesticides has been increasing (Briassoulis et al., 2001; Vontas et al., 2012). Additionally, owing to their short life cycle and high reproductive potential (Kim et al., 2014), aphids are not effectively controlled by current neonicotinoid-based insecticides, which contribute to environmental pollution (Foster et al., 2008; Puinean et al., 2010). Therefore, it is necessary to identify alternative means to prevent infections by *M. persicae* in order to reduce the use of chemical pesticides.

Insect growth regulators (IGR) that control the processes of insect metamorphosis and ecdysis are gaining attention, because of their low environmental toxicity, as eco-friendly pest control alternatives. Studies on various juvenile hormones and their receptors, which are a type of IGR, have been conducted (Smith, 1995; Palli and Retnakaran, 2001; Lee et al., 2015). Notably, the juvenile hormone receptor Methoprene-tolerant (Met), a transcription factor of the basic helix-loop-helix (bHLH) Per-Arnt-Sim (PAS) domain, has been identified in *Drosophila melanogaster*

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(Ashok et al., 1998; Wilson and Ashok, 1998). In order to activate the bHLH-PAS domain, bHLH-PAS proteins, known as Taiman, βFTZ-F1 interacting steroid receptor co-activator (FISC), or steroid receptor co-activator (SRC), and the juvenile hormone receptor bind to each other in the presence of a juvenile hormone (JH) to regulate the juvenile hormone-dependent genes (Lee et al., 2015).

In this study, plant extracts, with the potential to control peach aphids, were evaluated based on a screening system, using the transformant produced through a yeast two-hybrid assay with the juvenile hormone receptor of the peach aphid (Met) and the partner protein of the juvenile hormone receptor (SRC). Extracts with juvenile hormone disruptor (JHD) activity which could potentially be used to develop eco-friendly pest control agents were selected and sorted by plant groups.

Materials and Methods

Chemicals and plant extracts

Pyriproxyfen was purchased from Sigma-Aldrich (St. Louis, MO, USA). Plant diterpenes (methyl linderone) were isolated from *Lindera erythrocarpa*, as described in a previous study (Lee et al., 2015). Each reagent was prepared as a stock solution in dimethyl sulfoxide (DMSO) for the Yeast two-hybrid β-galactosidase assays (Y2H assays). Plant extracts (i.e., the methanol extracts of 1628 plant species) were received from the Korean Plant Extracts Bank (Daejeon, Korea).

Yeast two-hybrid β-galactosidase assays

The yeast two-hybrid binding test using quantitative β-galactosidase assay was carried out with the Y187 strain transformed with the JH receptor of *M. persicae* and its partner, Met-SRC, as described by Shin et al. (2018). The transformed Y187 strain was grown according to the protocol established by Shin et al. (2018). The optical density value of yeast (growth and treatment yeast cell) were read on a microplate reader (Spectramax 190, Molecular Devices Corporation, San Jose, CA) at a wavelength of 420 or 600 nm.

To screen the JHD activity of plants, 100 μL of grown yeast cells (OD_{600} = 0.2–0.3) were treated with both 0.1 ppm of pyriproxyfen and 10 ppm of each plant extract in 96-well plates. A positive control of 0.1 ppm of pyriproxyfen with 10 ppm of methyl linderone and a negative control of 0.1 ppm of pyriproxyfen with control solvent (DMSO) were included in each plate. The cells were incubated for 3 h and then subjected to an OD_{420} measurement, which yielded quantitative estimates of β-galactosidase activity. Subsequently, the OD_{420} values were normalized to an arbitrary unit of JHD. Methyl linderone was used as a positive control, owing to its strong interference with pyriproxyfen-mediated Met-SRC binding in tested *M. persicae* β-galactosidase assay systems (Shin et al., 2018). The binding interference by 10 ppm methyl linderone was calculated as a single arbitrary unit of JHD activity. The averages of duplicate experiments (in arbitrary units) were then used to calculate the specific JHD activity of each plant extract, using the following formula:

$$A = \frac{OD_{420}\ \text{Control} - OD_{420}\ \text{PE}}{OD_{420}\ \text{Control} - OD_{420}\ \text{ML10}}$$

where A represents the JHD activity (if A < 0, then A = 0), OD_{420} Control is the absorbance of yeast cells treated with JH, OD_{420} PE is the absorbance of yeast cells treated with 0.1 ppm of pyriproxyfen and 10 ppm of plant extract, and OD_{420} ML10 is the absorbance of cells treated with 0.1 ppm of pyriproxyfen and 10 ppm of methyl linderone.

Results and Discussion

JHD activities per plant groups

We tested 3648 plant extracts (from 167 families, including 1628 species), directly adding each extract to the yeast culture to investigate whether the pyriproxyfen-mediated Met-SRC binding could be disrupted by plant

Table 1. Summary of the screening of the juvenile hormone disruptor (JHD) activity of plant extracts

JHD activity	Number of plant extracts
JHD < 0.1	1,274
0.1 ≤ JHD < 0.2	391
0.2 ≤ JHD < 0.3	469
0.3 ≤ JHD < 0.4	438
0.4 ≤ JHD < 0.5	322
0.5 ≤ JHD < 0.6	264
0.6 ≤ JHD < 0.7	170
0.7 ≤ JHD < 0.8	110
0.8 ≤ JHD < 0.9	75
0.9 ≤ JHD < 1.0	58
1.0 ≤ JHD	77
Total	3,648

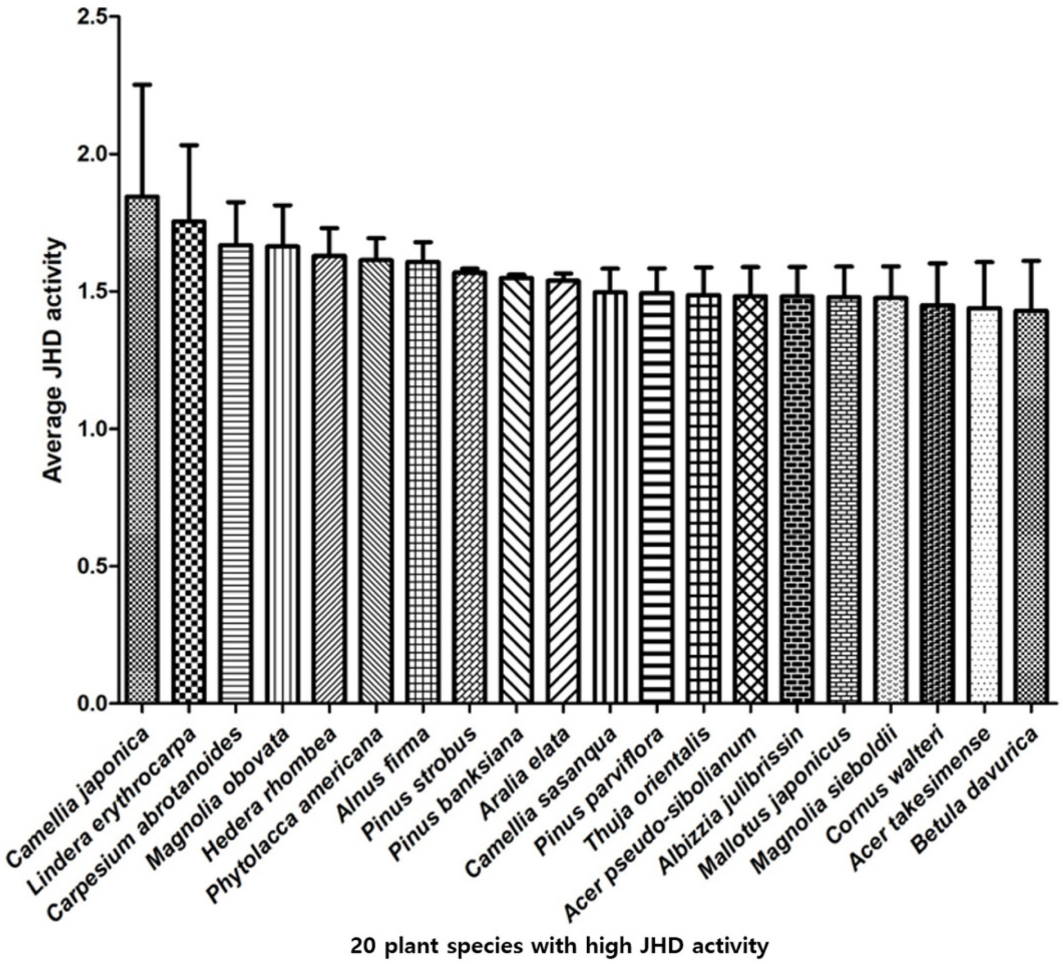

Fig. 1. Plant species with high juvenile hormone disruptor (JHD) activity among plant extracts evaluated as treatments against *M. persicae*. Values and error bars indicate means ± SD, respectively.

extracts in the two-hybrid yeast assay (Table 1). We selected the threshold for high JHD activity based on the results of a previous study (Oh et al., 2017). We set the high JHD value (JHD > 0.7) based on the JHD value (JHD > 0.5) indicated in the previous study (Oh et al., 2017). Among the plant extracts evaluated, in our study, 320 plant extracts were found to disrupt the pyriproxyfen-mediated binding of *M. persicae* to Met-SRC. We summarized the high JHD value (JHD > 0.7) based on the JHD value (JHD > 0.5) indicated in the previous study (Oh et al., 2017). Based on this, among 320 plant extracts, 272 (angiosperms) and 46 (gymnosperms) extracts were identified (Supplementary Table 1). The 20 most active extracts were identified and selected, and the extract from *Camellia* (Theaceae) showed the highest JHD activity (Fig. 1). Furthermore, most of the plants whose extracts showed superior JHD activity corresponded to gymnosperms and angiosperms (Fig. 2).

Confirmation of JHD activity per plant group

Extracts from the four major vascular plant groups, namely the Pteridophyta, Lycopodiophyta, gymnosperms, and angiosperms, had significant effects on *M. persicae* two-hybrid yeast assay. We found that the JHD activity was particularly elevated in gymnosperms, with a mean value significantly higher than that of the Pteridophyta, Lycopodiophyta, and angiosperms, respectively (Fig. 2). Furthermore, after determining the average JHD activity for Pteridophyta, Lycopodiophyta, and gymnosperm extracts, excluding less than 5 extracts, the top 10 plants were selected and identified from more than five extracts of angiosperms (Fig. 3). High JHD activity was evaluated from the extracts of two gymnosperm families, namely Cupressaceae, which contained the species *Platycladus orientalis*, and Pinaceae, which included pines (Fig. 3A), as well as for the Lycopodiophyta family Selaginellaceae (Fig. 3D), and Dryopteridaceae (Pteridophyta; Fig. 3C). In

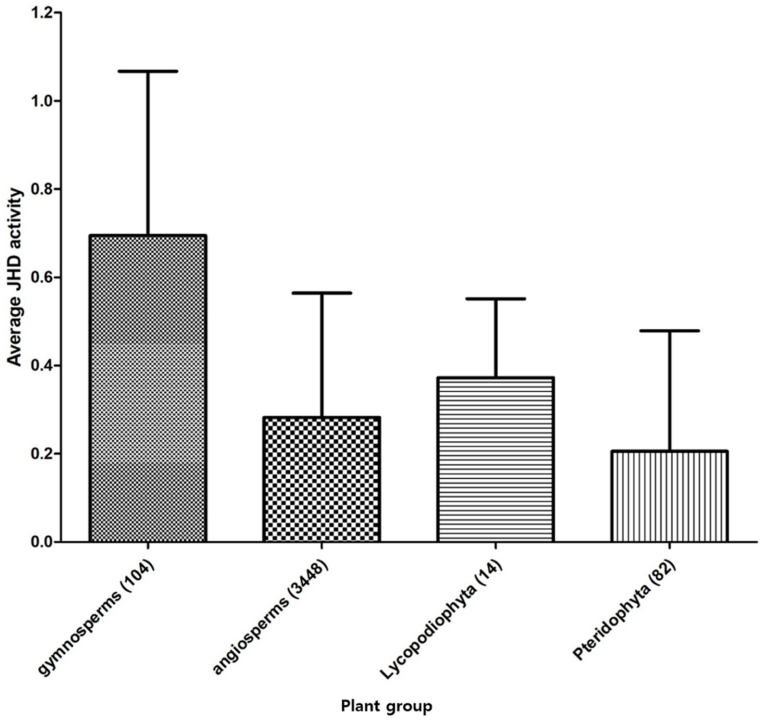

Fig. 2. Average juvenile hormone disruptor (JHD) activity values per plant groups. Values and error bars indicate means ± SD, respectively (Numbers in parentheses indicate plant extract).

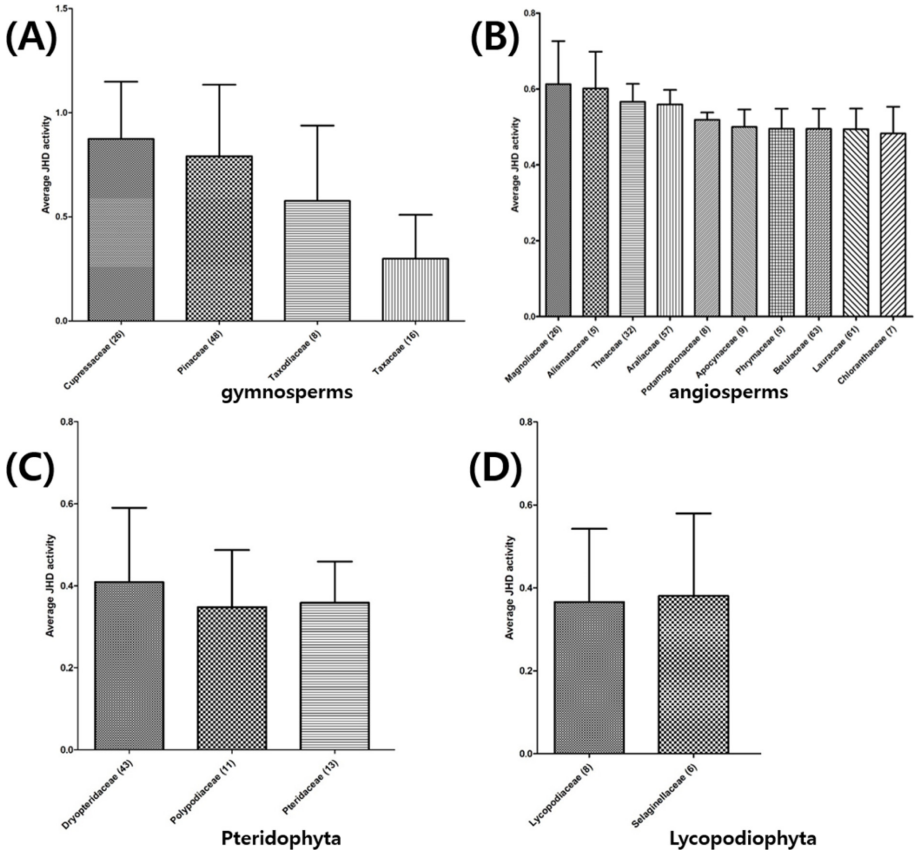

Fig. 3. Average juvenile hormone disruptor (JHD) activity values per plant family, subdivided in plant group. (A) gymnosperms; (B) angiosperms; (C) Pteridophyta; (D) Lycopodiophyta. Values and error bars indicate means ± SD, respectively. (Numbers in parentheses indicate plant extract)

addition, JHD activity was also measured in angiosperm, namely Magnoliaceae and Alismataceae (Fig. 3B). The JHD activity of both Cupressaceae and Pinaceae was higher than that of other plant taxa and higher than the average value for all plant extracts. Also, this study confirmed that the effect of JHD on the *M. persicae*-specific JHD assay system differs according to plant group. We found that the JHD activity was particularly concentrated in the gymnosperms (Oh et al., 2017); the mean JHD activity of the gymnosperm extracts was significantly higher than that of the Lycopodiophyta, Pteridophyta, and angiosperms, respectively.

In addition, camellidin II from the camellia flower, which was shown to have high JHD activity in this study, is known to be an antifeedant for *Eurema hecabe* larvae (Numata et al., 1987). Secondary metabolites which are produced by plants, such as camellidin II, are known to react against insects that harm plants and to elicit the production of substances similar to the hormones of those insects (Toong et al., 1988; Dinan, 2001). Accordingly, plant extracts containing secondary metabolites can potentially be developed as novel insecticides that are safe and environment-friendly. Notably, the present screening method with plant extracts can rapidly identify candidates for new insecticide substances. Therefore, our results indicate that the discovered JHD compounds could be used to develop novel insecticides.

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Conflict of Interest

The authors declare that there are no conflicts of interest

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