

Repellent Effects of Volatile Extracts from Herb Plants against Larvae of *Pieris rapae crucivora* Boisduval

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Received: November 14, 2011

Accepted: December 6, 2011

Online Published: April 1, 2012

doi:10.5539/jas.v4n5p145

URL: <http://dx.doi.org/10.5539/jas.v4n5p145>

Abstract

Environmental pollution and intensive use of toxic chemicals, as well as resistance to pesticides, has forced scientists to find new ways of controlling pests. Numerous substances that repel insects and inhibit eating and egg-laying have been discovered in a variety of plants. The cabbage white butterfly, *Pieris rapae crucivora* Boisduval, is a serious pest to Brassicaca species such as cabbage, cauliflower and broccoli, and its larvae damage the host plant leaves when feeding. In this study, to develop a botanical insecticide against *P. rapae* larvae, we assessed the effect of 7 kinds of herb (rosemary, spearmint, eucalyptus, sage, chili pepper, chives and tansy) extracts as feeding repellents against *P. rapae* larvae. Feeding repellent tests were conducted by placing two cabbage leaf disks on filter paper treated with herb extract or diethyl ether in a box; then a larva was placed between the disks and its preference was observed. The repellent rate of rosemary, spearmint, eucalyptus, sage, chili pepper, chives and tansy was 75%, 72%, 64%, 59%, 40%, 40% and 36%, respectively. These results clarified that of the tested herbs, rosemary and spearmint volatile extracts have a notable feeding repellent effect against *P. rapae* larvae.

Keywords: *Pieris rapae*, Larvae, Feeding repellent

1. Introduction

There are growing concerns on the use of chemical pesticides and fertilizers due to environmental pollution, adverse effects on human health, disruption of natural biological control and evolution of pesticide-resistant pest populations. As consumer interest in the safety of food and agricultural products has risen in recent years, producers are increasingly focused on pesticide-free and organic farming that take human health and the environment into account. In Japan, about 600 agricultural pesticides have been included in the Positive List since its introduction in 2006. This list details the maximum limits of chemical residues permissible in food products. Without the use of chemicals, however, farmers risk disease and insect damage to crops (Perry *et al.*, 1998; Isman, 2006). Botanical insecticides are an environmentally friendly alternative to hazardous chemicals as they are plant-derived insecticides which occur either naturally or are extracts of such plants (Gupta *et al.*, 2005)

Many plant species are known to possess insecticidal properties, and the compounds from these plants have a number of useful activities such as toxicity, repellency, feeding and oviposition deterrence and insect growth regulation (Sharma and Gupta, 2009). In mixed vegetation, odors released by some plants can mask the effect of odors released by other plants (Cromartie, 1975), thereby disorientating insects such that they spend less time on plants suitable for egg-laying and feeding (Lamb, 1989). The cabbage white butterfly, *Pieris rapae crucivora* Boisduval, is a serious pest of Brassicaceae plants such as cabbage, cauliflower and broccoli (Ikeura *et al.*, 2010), and its larvae damage the host plant leaves when feeding (Verschaffelt, 1911).

Gao *et al.* (2004) showed that deoxypodophyllotoxin, indentified in *Juniperus Sabina* L., has insecticidal activity against *P. rapae* larvae. In addition, Zhong *et al.* (2006) showed that rhodojaponin-III isolated from *Rhododendron molle* G. Don flowers is an antifeedant, stomach poison, contact toxicant and growth inhibitor against *P. rapae*. However, the compounds indentified in their study were non-volatile. Only a few reports have been published on the feeding repellent effect of volatile extracts against *P. rapae* larvae. In this study, we investigated the feeding repellent effect of 7 kinds of herb extracts to develop a botanical insecticide against *P. rapae* larvae.

2. Materials and Methods

This study was conducted at the laboratory and field of Meiji University from September 20th to December 20th 2007.

2.1 Plants

The following 7 plants, well known by agriculturists and horticulturists, and common in the literature, were selected as the basis for herbal extracts: rosemary (*Rosmarinus officinalis* L.), spearmint (*Mentha spicata* L.), eucalyptus (*Eucalyptus globules* L.), chives (*Allium schoenoprasum* L.), sage (*Salvia officinale* L.), tansy (*Tanacetum vulgre* L.) and chili pepper (*Capsicum annum* L.).

2.2 Insects

Gravid female adults of *P. rapae crucivora* Boisduval were collected from cabbage patches in Kawasaki city. They were released in a cage (1.6 m × 1.6 m × 1.8 m) made from white shading net in a greenhouse of Meiji University, fed an approximately 10% sucrose solution and were free to oviposit on potted cabbages. When eggs were laid, the cabbages were placed under a natural condition in the greenhouse until hatching. The hatched larvae, which were fed cabbage in a plastic case (10 cm × 20 cm × 7 cm), were reared at 25 °C under a 16L:8D regime until being at the fourth instar.

2.3 Herb Extraction

Ten grams of fresh leaves were homogenized and mixed with 40 ml of diethyl ether at 4 °C for 4 h with a stirrer. Next, the sample was centrifuged at 12000 rpm at 4 °C for 20 min and filtered, and the filtrate was used as the herb extract.

2.4 Feeding Repellent Assay

A feeding repellent assay was conducted on two cabbage leaf disks (3 cm × 3 cm) on filter paper (5 cm × 5 cm) treated with herb extract or diethyl ether in a box with dimensions of 20 cm × 15 cm × 8 cm. A larva was placed between the disks, and the filter paper chosen by the larva was noted. Thirty larvae were used for each treatment. The repellent rate is expressed as follows: number of larvae choosing treated paper / (number of larvae choosing treated paper + number of larvae choosing control paper) × 100.

3. Results

The repellent effect of each extract is shown in Fig. 2. Only rosemary (75%; chi-test, $\chi^2=14.75$, $p<0.001$) and spearmint (72%; $\chi^2=11.36$, $p<0.001$) extracts had a significant feeding repellent effect against the larvae.

4. Discussion

Plants have wide action spectra, whereas a particular plant can affect insects though different mechanisms of action. In this study, rosemary and spearmint affected *P. rapae* larvae by inhibiting their feeding, suggesting the presence of feeding repellent compounds in the volatile extracts.

To date, there have been many studies on repellency and the insecticidal effects of herbs against pests (Dover, 1985; Jbilou, *et al.*, 2006; Sharma and Gupta, 2009). Rosemary is known to have a repellent effect on various pests, and Horii (1998) and Sampson *et al.* (2005) reported that rosemary oil repelled the green peach aphid, *Myzus persica* and *Lipaphis pseudobrassicae*. Waliwitiya *et al.* (2009) assessed the essential oil of rosemary against larval stages of *Aedes aegypti* and found that the larvicidal activity of 5 terpenoids, pulegone, thymol,

eugenol, trans-anethole, and citronellal exhibited high larvicidal activity against *A. aegypti*. Dover (1985) reported that the alcohol extract of rosemary has a repellent effect on *P. brassicae*. Moreover, mint oil is known to repel various pests and insects (Sampson, *et al.*, 2005; Abbaszadeh, *et al.*, 2009). However, there is no data on the antifeeding effects of rosemary on *P. rapae* larvae; nevertheless rosemary and spearmint are known to repel various pests.

P. brassicae belongs to the same family as *P. rapae* and avoids feeding on leaves treated with hyssop, rosemary, sage, thyme, white clover and eucalyptus (Dover, 1985; Sharma and Dupla, 2009). On the other hand, *P. rapae* larvae avoid feeding on the wild mustard, *Erysimum cheiranthoides* (Sachdev-Gupta *et al.*, 1993). Thus, *P. spp.* reject various plants, these repellents are soluble. The findings of the present study suggest that compounds from rosemary may be volatile due to extract high volatile organic solvent. Furthermore, it seems desirable to use rosemary for companion plants.

In the future, we need to identify the feeding repellent compounds in rosemary. In conclusion, volatile extracts of rosemary and spearmint have a strong feeding repellent effect on *P. rapae* larvae.

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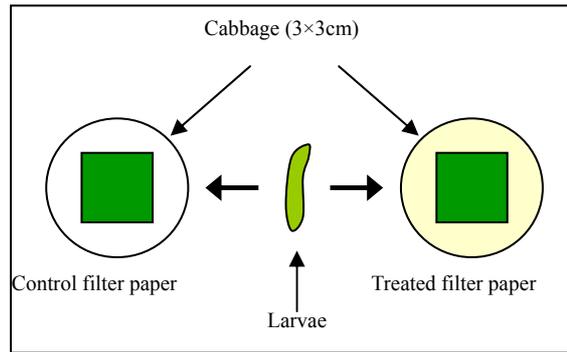


Figure 1. Preference of larvae to the cabbage leaf on treated filter paper (5 cm in diameter) or the control

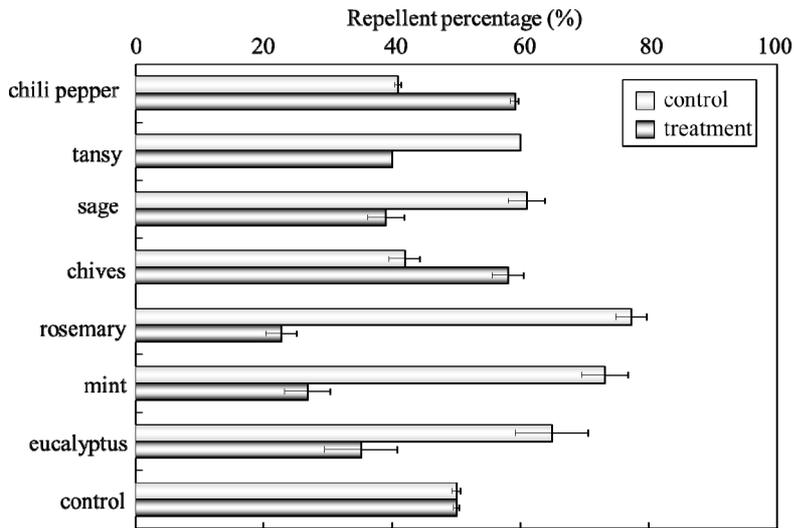


Figure 2. Repellent effect of 7 kinds of herb extracts against *P. rapae* larvae

Error bar indicates standard error (n=10). The repellent rate was calculated as: (number of larvae that selected control filter paper) / (number of larvae that selected treated filter paper + number of larvae that selected control filter paper) × 100.