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The efficacy and translocation behavior of carabrone in wheat and cucumber

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ABSTRACT

Carabrone, which has high antimicrobial activity and broad disease prevention spectrum, is expected to be developed as a new type of plant source fungicide. In the current study, the absorption and translocation behavior of carabrone in plants were clarified Potted monocotyledonous wheat and dicotyledonous cucumber were chosen as the tested plants. The results showed that carabrone at 1000 mg/L exhibited over 65% efficacy against wheat and cucumber powdery mildew when applied by foliar spray or irrigation, and over 60% and 45% efficacy obtained against wheat take-all and cucumber fusarium wilt respectively when applied by irrigation. In contrast, no efficacy was observed against the two root diseases when carabrone was applied by foliar spray. Moreover, the absorption and translocation behavior of carabrone in wheat and cucumber were further determined by the high-performance liquid chromatography (HPLC) method. The result showed that carabrone in both soil and water could be absorbed and transported upward readily by wheat and cucumber, especially in water. However, no carabrone was detected in the root samples within 72 h when it was sprayed on the leaves of wheat and cucumber. The results suggest that carabrone could be readily absorbed by the roots of wheat and cucumber and transported upward but could not be absorbed by their leaves and transported downward. This information could provide guidance for field use and increase our understanding of the action mechanism of carabrone.

1. Introduction

Plant diseases seriously limit food production at varying levels worldwide each year (Brãse et al., 2009; Haverkort et al., 2008; Shimizu et al., 2009). According to the statistics of World Food and Agriculture Organization, world food production is reduced by 10% because of the diseases. At present, chemical control remains the main method for plant disease management. However, because of the long-term use of chemical pesticides, there are increasingly serious problems such as pesticide resistance, chemical residues and threats to human safety (Akila et al., 2011; Jiang et al., 2006; Paster and Bullerman, 1988). Thus, developing alternative pesticides with novel modes of action, which are safe to the environment and humans, is urgently necessary. Owing to advantages in environmental and human health safety, as well as management of resistance development, botanical pesticides especially extracted from plants have become of greater interest for development and registration (Pitarokili et al., 2003; Thangavelu et al., 2004; Tripathi and Dubey, 2004).

Carabrone, extracted from *Compositae Carpesium* L. plants, belongs to the carabrane type sesquiterpene lactone compound family, and is a characteristic natural product of compositae plants (Holub et al., 1972; Sevil, 1992; Wang et al., 2009; Zdero et al., 1983). Previous studies have shown that sesquiterpene lactones exhibit excellent anticancer, anti-inflammatory and antifungal activities, which are widely used in medical and agricultural fields (De Ford et al., 2015; Macías et al., 2006; Olivaro et al., 2016; Wu et al., 2016). Our previous work showed that carabrone has good inhibitory activity on both the growth of mycelia and spore germination

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of a series of plant pathogens such as *Phytophthora capsici* Leonian, *Gaeumannomyces graminis* var. *Tritici,* and *Colletotrichum lagenarium* (Feng et al., 2002; Ren et al., 2012). Therefore, carabrone has the potential to be used as an alternative botanical pesticide for disease management. However, little is known about the absorption and translocation characteristics of carabrone in plants.

The objectives of this study were to (a) evaluate the control efficacy of carabrone in greenhouse experiments against wheat and cucumber powdery mildew, and wheat take-all and cucumber fusarium wilt; (b) determine the absorption and translocation behavior of carabrone in wheat and cucumber by the HPLC method; and (c) determine the absorption and translocation content of carabrone in water and soil by wheat and cucumber. These results will provide new information for farmers in the field use of carabrone in future and increase our understanding of the mechanism of action of carabrone.

2. Materials and methods

2.1. Chemicals, strains, plants, and other material

Carabrone (purity≥96%) and 10% carabrone microemulsion were supplied by the Research & Development Center of Bio-rational Pesticide, Northwest A & F University. The following reference fungicides were used: 15% triadimefon (WP, Sichuan Guoguang Agriculture Fertilizer Co., Ltd.) and 50% carbendazim (WP, China Weifang Ruizefeng Agriculture Fertilizer Co., Ltd.).

The following recompounds were used: methanol (analytically and chromatographically pure), acetonitrile (chromatographically pure), acetone (analytically pure), dimethylbenzene (analytically pure), and emulsifier Tween-80.

Tested fungal strains *Blumeria graminis, Sphaerotheca fuliginea*, *Gaeumannomyces graminis* var. *tritici,* and *Fusarium oxysporum* f. sp. *cucumerinum* were supplied by the Research & Development Center of Bio-rational Pesticide, Northwest A & F University. Varieties of wheat (Landrace Huixianhong variety) and cucumber (Zhongnong No.8 variety), known to be susceptible to the diseases to be evaluated were purchased from Yangling Seed Market, Shaanxi Province of China.

C_{18} solid-phase extraction column was used for purification.

2.2. Efficacy of carabrone in greenhouse against four plant diseases

2.2.1. Efficacy of carabrone against wheat and cucumber powdery mildew

Wheat and cucumber seeds were sowed in plastic flower pots (10 cm in diameter) filled with sterilized soil. After 2 weeks, wheat and cucumber seedlings with 2–3 leaves were sprayed or irrigated (10 mL per treatment) with either water, carabrone at 500 mg/L and 1000 mg/L, or triadimefon at 150 mg/L. Inoculation and disease incidence evaluation were conducted according to Wang's method (Wang et al., 2005). Briefly, fresh spores of powdery mildew (1×10^5/m) produced within 24 h were uniformly sprayed on the leaves of wheat and cucumber seedlings. Seven to 10 days after inoculation, disease incidence was calculated as follows: 0 = no visible symptoms; 1 = lesion on < 5% of the leaf area; 3 = lesion on 6–15% of the leaf area, but few conidia; 5 = lesion on 16–30% of the leaf area, colonies with well-developed hyphae and abundant conidia, but colonies not joined together; 7 = lesion on 30–50% of the leaf area, and 9 = lesion on >50% of the leaf area, colonies with well-developed hyphae, abundant conidia, and colonies mostly joined together.

For the efficacy against cucumber powdery mildew, the fungicides were sprayed or irrigated as described earlier when the cucumber shows growth of 1 or 2 true leaves. The disease incidence was evaluated 7 days after inoculation as described by Liang et al. (2005) with a rating (r) of 0, 1, 3, 5, 7, or 9, denoting proportions of disease over the whole leaf area of 0, <1, 2–5, 6–20, 21–40, and >40%, respectively. The disease index was calculated as follows:

$$\text{Disease index} = [n \times (1) + n \times (3) + n \times (5) + n \times (7) + n \times (9)]/\text{number of all detected leaves} \times 9$$

For protective activity tests, plants were treated with the compound 1 day before inoculation. For curative activity tests, plants were treated with the compound 1 day after inoculation. Each treatment had four replicate pots placed in a completely randomized design, and the experiment was repeated twice.

2.2.2. Efficacy of carabrone against wheat take-all

The efficacy of carabrone against wheat take-all was calculated using Peterson's method (Peterson and Edgington, 1971). Wheat seeds were sowed as described in section 2.2.1. Mycelial plugs (5 mm in diameter) collected from the margin of the colony of *G. graminis* were mixed with the soil at about 1–2 cm deep below the roots of plants. Five mycelial plugs per pot were used, and each plant was irrigated with either 10 mL of water, carabrone at 500 mg/L and 1000 mg/L, or triadimefon at 150 mg/L. Protective and curative activities were tested as described in section 2.2.1. The strains were then placed in greenhouse with 80% relative humidity. After 3 weeks, the disease incidence was evaluated on the basis of disease grading index referring to Bithell's method (Bithell et al., 2016). Each treatment had four replicates and the experiment was conducted twice.

2.2.3. Efficacy of carabrone against cucumber fusarium wilt

Cucumber seeds were sowed as described in section 2.2.1. Each pot was irrigated with 10 mL spore suspension (1×10^5/mL). Protective and curative activities and the concentrations of carabrone were the same as described in section 2.2.1. Carbendazim at 200 mg/L was used as the reference fungicide. The disease incidence was evaluated on the basis of the disease grading index referring to He's method (He et al., 2015). Each treatment had four replicates and the experiment was conducted twice.

Data from repeated experiments in this study were combined for analysis because variances between the experiments were homogeneous. All data were processed with the analysis of variance (ANOVA) using SPSS 14.0 (Statistical Package for the Social Science, SPSS Inc., Chicago, IL). When the ANOVA was significant ($P = 0.05$), the mean values were separated using Fisher's protected least significant difference.

2.3. Analysis the absorption and translocation activity of carabrone by HPLC

2.3.1. Absorption and translocation of carabrone in soil by wheat and cucumber

Wheat and cucumber cultured as above were irrigated with 10 mL carabrone at 400 mg/L. The wheat and cucumber were cultured under natural light in greenhouse (25 °C, 80% relative humidity). The aboveground tissues were cut off at 4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 h, and four pots per treatment were used. The tissues were then rinsed with water, air dried, weighed and kept at −20 °C.

2.3.2. Absorption and upward translocation of carabrone by wheat and cucumber in water

Wheat and cucumber were cultured as described above and allowed to grow 2 to 3 or 1 to 2 true leaves respectively. Plants were then dug out, rinsed with water, and inserted into 250 mL conical