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Research Paper

Comprehensive evaluation of effective constituents in total alkaloids from *Sophora alopecuroides* L. and their joint action against aphids by laboratory toxicity and field efficacy

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ABSTRACT

Alkaloids are the main constituents that contributed to the insecticidal activity of plant *Sophora alopecuroides* L. (Fabaceae). Using an acid extraction and alkali precipitation method, total alkaloids were extracted and then six alkaloids were isolated, which were identified as matrine, oxymatrine, aloperine, cytisine, sophocarpine and sophoridine. To characterize the aphicidal spectrum and joint action of these compounds, their contact toxicity and joint action against aphids were evaluated by bioassay and field trial. Results showed that all the test alkaloids had high and broad spectrum aphicidal activities, but the toxicities varied significantly among alkaloids with different aphid species. Based on contact toxicity, cytisine was the most active alkaloid among six monomers. In the meantime, it was confirmed that the combination of cytisine, aloperine, oxymatrine and sophocarpine showed the most significant synergistic effect against *A. craccivora*, with the poison ratio of 1.62. Furthermore, field trials on four aphid species also indicated that the quaternary alkaloids possessed a more significant efficacy than others, with the efficacy about 80% on the 7th day post application. Thus, both bioassay and field trial proved that the combination of the four monomers possesses strikingly aphicidal synergistic action. As environmental friendly natural products, *S. alopecuroides* alkaloids can serve as potential, broad spectrum aphid-control agents, and the four alkaloid monomers would be important quality parameters for development and application of pesticide products from *S. alopecuroides* alkaloids.

1. Introduction

Aphids (Hemiptera: Aphididae) are important insect pests of a wide range of host plants, and are widely distributed herbivorous insects accounting for more than 4300 described species (Francis et al., 2006; Gibbs et al., 2010). They can cause damages to crops through directly feeding on plants, transmitting plant pathogenic viruses, and secreting honeydew to result in secondary fungal growth and inhibiting photosynthesis (Dedryver et al., 2010; Edwards et al., 2008). The combination of reproductive features and specific feeding habits make aphids one of the most economically important groups of pest in agriculture (Guerrieri and Digilio, 2008). Annual worldwide economic losses due to the damage of aphids are estimated at hundreds of millions of dollars (Larsson, 2005).

In the past few decades, aphids have been controlled by the application of almost all classes of chemical insecticides, including carbamates, organophosphorous, pyrethroids and neonicotinoids. As a result of frequent chemical application, aphids developed high resistance to numerous commonly used insecticides in many agricultural areas (Gore et al., 2013; Silva et al., 2012; Will and Vilcinskas, 2013).

Botanical pesticides with complex and specific mechanisms showed its advantages in reducing the pest resistances and economical stress from synthesized chemical pesticides (Isman, 2006). For example, neem seed oil (NSO) and neem seed extract (NSE) were demonstrated to be effective aphicides in laboratory and field trials (Kraiss and Cullen, 2008; Shah et al., 2017). Moreover, as reported, extracts from *Rhamnus dispermus* (Rhamnaceae) providing valuable mortality rates for the peach trunk aphid *Pterochloroides persicae* (Hemiptera: Lachnidae) could be used as botanical insecticides in the integrated pest management programs (Ateyyat and Abu-Darwish, 2008). In fact, plant extracts have long been a subject of researches aiming at developing alternatives to synthetic chemical insecticides.

Sophora alopecuroides (Fabaceae), a perennial herb, is widely distributed in the northwest of China. It has been demonstrated that the principal bioactive constituents of *S. alopecuroides* are alkaloids (Lu et al., 2014), which exhibit a wide range of activities, including insecticidal, antiviral, fungicidal, and herbicidal effects (Akhtar et al., 2012; Zhang et al., 2017), among which more researches are focused on

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its insecticidal activity. *S. alopecuroides* alkaloids are effective to *Brontispa Longissima* (Coleoptera: Hispidae), *Leucania separate* (Lepidoptera: Noctuidae) (Cai et al., 2009), *Plutella xylostella* (Lepidoptera: Tineidae), *Helicovera armigera* (Lepidoptera: Noctuidae) (Cai et al., 2004), *Aphis laburnis* (Hemiptera: Aphididae) (Goggin, 2007), *Aphis* sp. (Hemiptera: Aphididae) (Han et al., 2009) and *Plagiodera versicoloraetc* (Coleoptera: Chrysomelidae) (Ma et al., 2006). Phytochemical studies showed that there were a variety of alkaloids in *S. alopecuroides*, including sophocarpine, matrine, sophoramine, sophoridine, aloperine, cytisine, oxymatrine, etc. (Song et al., 1999; Wang et al., 2012). However, what constituents in total alkaloids should be responsible for the bioactivity are not completely clear yet.

In addition, there are often complicated interactions among plant secondary metabolites. Identifying their joint insecticidal actions would be meaningful for development of botanical pesticides (Tak et al., 2016). As an important insecticidal plant, clarification of joint insecticidal actions among alkaloid monomers in *S. alopecuroides* would be very important for commercial application as a botanical pesticide.

Approximately 100 aphid species have successfully exploited agroecosystems to become serious economical pests (Landis et al., 2000). However, except *Lipaphis erysimi* and *Aphis sp*., activities of single alkaloid from *S. alopecuroides* against other species of aphids have not been affirmed yet. Also, little research in *S. alopecuroides* alkaloids controlling aphids in field has been reported. To assess the effective constituents and the potential of *S. alopecuroides* alkaloids for aphids management, total alkaloid and monomer alkaloids were isolated, and aphicidal activities and their joint action were evaluated by both bioassay and field trial against aphids, including *Myzus persicae* Sulzer (Hemiptera: Aphididae), *Aphis craccivora* Koch (Hemiptera: Aphididae), *Aphis citricola van der* Goot (Hemiptera: Aphididae), *Macrosiphum rosirvorum* Zhang (Hemiptera: Aphididae), *Sitobion avenae* Fabricius (Hemiptera: Aphididae), *Brevicoryne brassicae* Linnaeus (Hemiptera: Aphididae) and *Aphis* sp. (Hemiptera: Aphididae).

2. Materials and methods

2.1. Test alkaloids

The test alkaloids for indoor and outdoor experiments were isolated from *Sophora alopecuroides* plants, which were collected in Otog Front Banner of Inner Mongolia, China. The plants were identified by Dr. Li Yan, College of Life Sciences, Northwest A & F University and then air-dried.

2.2. Test aphids

Myzus persicae, *A. craccivora and S. avenae* were reared in the greenhouse at 22 ± 2 °C, 16:8 L/D, and 50 ± 10% RH in Northwest A & F University.

Aphis citricola van der adults were collected from apple trees in the pilot demonstration base at Horticulture School of Northwest A & F University.

Macrosiphum rosirvorum adults were collected from Chinese roses (*Rosa chinensis* Jacq) in the campus of Northwest A & F University.

Brevicoryne brassicae adults were picked in the cabbage garden at the suburb of Yangling Town.

Aphis sp. adults were collected from matrimony vine (*Lycium chinense*) garden in Zhongning County of Ningxia Hui Autonomous Region.

In bioassay, similar size of healthy and wingless adult aphids were selected for test.

2.3. Extraction and isolation

2.3.1. Extracts and preparation of the crude alkaloidal fraction

The dried aerial part of *S. alopecuroides* (10 kg) were crushed and then extracted thrice (3 days per time) with MeOH at room temperature. The filtrate was concentrated in vacuo at 45 °C, and the residue (598.3 g) obtained was stored at 4 °C, then, suspended in 3% aqueous tartaric acid. The acidic extract was washed with EtOAc to give neutral components (152.6 g). The aqueous acidic fraction was then made basic with NaOH (pH 10) and extracted again with EtOAc until the aqueous layer was free of alkaloids. The combined EtOAc extracts were evaporated in vacuo to yield the crude alkaloidal fraction as dark brown residue (29.7 g).

2.3.2. Fractionation of crude extracts and isolation of active constituents

The crude alkaloidal extract was chromatographed on a silica gel column (10 × 120 cm, 360 g) and successively eluted with a stepwise gradient of $CHCl_3$-CH_3COCH_3 (99: 1, 85: 15, 80: 20, 50: 50, 1: 99, each 1.7 L) to afford five subfractions (Fr. 1–5). Of which, Fr. 2 was loaded on a reversed-phase column (RP-18) eluting with a gradient of MeOH-Water (10: 90, 30: 70, 50: 50, 70: 30, 90: 10, 100: 0) to obtain six subfractions (Fr. 2-1- Fr. 2–6), Fr. 2–3 (1.20 g) was separated by semipreparative HPLC using CH_3OH-H_2O (80: 20, 5.0 mL/min) as the mobile phase, yielding pure compound **1** (52.3 mg), **2** (19.4 mg) and **3** (17.1 mg). Fraction 2–4 (1.6 g) was subjected to preparative HPLC (MeOH/H_2O, 60:40, v/v) to obtain compound **4** (42.7 mg). Fr. 3 (4.9 g) was divided into 5 fractions (Fr. 3-1–3-5) by preliminary isolation of a silica gel column (Pe-CH_3COCH_3, 100:0 to 0:100, v/v). Of which, Fr. 3–2 (421.0 mg) was subjected to preparative HPLC (MeOH/H_2O, 70:30, v/v) to obtain compound **5** (24.0 mg). Fr. 3-3 (936 mg) was subjected to preparative HPLC (MeOH/H_2O, 60:40, v/v) to obtain compound **6** (26.4 mg).

2.4. Bioassay

The aphicidal activity of the alkaloids from *S. alopecuroides* was tested by conventional topical application method with capillary micro burette against seven species of aphids (Hewlett and Lloyd, 1960). Each dose, dissolved in acetone (80%) was added on the pronotum of aphid with 0.06 μL dropper. For each dose, the similar size of healthy and wingless adult aphids were treated and then placed on three Petri dishes (9 cm in diameter) at 25 ± 2 °C and 50 ± 10% RH. Thirty aphids were tested in each treatment, and each treatment was repeated three times. Mortality was recorded 24 h after treatment and the medial lethal dose (LD_{50}) values were analyzed.

2.5. Joint action of the alkaloid monomers from S. alopecuroides

Each alkaloid monomer at the concentration of LD_{50} was prepared. Combinations of every 2 in the 6 monomers were prepared at LD_{50} ratio of 1:1 (V: V). Then joint actions against *A. craccivora* were determined with the bioassay method mentioned above.

The joint actions against *A. craccivora* of every 3, 4 and 5 in the 6 alkaloid monomers mixed with the LD_{50} volume ratio of each monomer of 1:1:1, 1:1:1:1, and 1:1:1:1:1 (volume ratios of LD_{50}) were measured with the same method above, respectively.

2.6. Plot experiment and field trials

2.6.1. Aphis craccivora

The plot experiment was conducted on broad bean plants (*Vicia faba* L.) in a greenhouse (Yangling, Shaanxi Province, China) in March 2017. Broad beans were sown in February. Twenty days after seeding, *A. craccivora* aphids reared in the incubator were transferred on seedlings and maintained for several days in greenhouse. When the number of individuals on each plant was more than fifty, these plants were used for the test. Each treatment with three replicates covered an area of 2.5 square meters.

2.6.2. Aphis citricola van der

In May 2017, the field trials were carried out in an apple orchard