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Nematicidal activity of *Glycyrrhiza uralensis* Fisch. root extracts on *Meloidogyne incognita* eggs and juveniles

Dang-Minh-Chanh Nguyen[1,3] · Thi-Hoan Luong[2] · Van-Viet Nguyen[3] · Woo-Jin Jung[1]

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Abstract This study evaluated the *in vitro* nematicidal activity of *Glycyrrhiza uralensis* root extracts (GuRE) on *Meloidogyne incognita* eggs and juveniles. The results showed that treatment of *M. incognita* eggs with 2.0 mg/mL GuRE for 5 and 10 d resulted in 64.0 and 68.1% hatch inhibition, respectively. Furthermore, the relative mortality of J2 was 96.2% after treatment with 2.0 mg/mL GuRE for 48 h. Changes in the shape of the eggs and juveniles were determined after incubation with 2.0 mg/mL GuRE for 5 d and 48 h, respectively. These preliminary results suggest that GuRE can be used as an environment-friendly bio-nematicide to control root-knot nematodes. In the future, *in vivo* assays should be conducted using GuRE to ascertain its potential for widespread application as a nematicide.

Keywords Hatching · Juvenile · Medicinal plant · *Plant-parasitic nematodes*

Woo-Jin Jung (✉)
E-mail: woojung@jnu.ac.kr

Dang-Minh-Chanh Nguyen (✉)
E-mail: ndmchanh75@gmail.com; minhchanh260875@jnu.ac.kr

[1]Department of Agricultural Chemistry, Institute of Environmentally Friendly Agriculture (IEFA), College of Agriculture and Life Sciences, Chonnam National University, 77 Yongbong-ro, Buk-gu, Gwangju 61186, Republic of Korea

[2]National Institute of Medicinal Materials (NIMM), Hanoi 11000, Vietnam

[3]Research and Development Institute for Agriculture (Nafoods), Hanoi 11000, Vietnam

Introduction

Plant-parasitic nematodes (PPN) cause severe losses in agricultural yield and significantly impact disease complexes involving other pathogenic organisms. Infected plants show typical symptoms such as root galling, stunting, nutrient deficiency, and, in particular, nitrogen deficiency. The annual economic losses caused by PPN in major staple crops were estimated to be 215 billion USD [1]. Among endoparasitic nematodes, *Meloidogyne incognita* is a nematoderoot-knot nematodes (RKN) in the family Heteroderidae. It is one of the four most common species worldwide and has numerous hosts. This nematode typically incites large, usually irregular galls on roots as a result of parasitism. It is estimated that more than 3,000 plant species can be affected by this nematode [2,3]. These plant-parasitic nematodes have a broad spectrum of hosts. They can survive in soil without a host plant and persist for extended periods even under extremely dry conditions [4].

Various control measures, including the destruction of diseased plants, the use of sanitary measures, disease-free tissue culture planting material, and tolerant varieties as well as other integrated management methods, have been utilized to manage this disease. In particular, several effective chemical nematicides have been recommended for use against RKNs, but they are not considered to be long-term solutions due to concerns regarding expense, exposure risks, toxic residues, and environmental hazards [5-8]. Therefore, alternative control methods that can be safely and effectively used are urgently required [9,10]. Plant preparations are potential alternatives because they degrade into non-toxic products and have fewer side effects on non-target organisms and within the broader environment. Additionally, such agents often act at multiple and novel target sites, thereby reducing the potential of plant-parasitic nematodes becoming resistant to them [2,10].

Glycyrrhiza uralensis Fisch. is a perennial medicinal plant that is native to China, Mongolia, Russia, and Korea [11]. It grows mostly in arid and semi-arid desert grasslands, desert edges, and

loess hilly areas [12]. The plant prefers a dark, humid, dry climate with long sunshine and low temperature [12]. Its root is 1-3 cm in diameter with brown skin and light yellow inside and it taste sweet. The plant is very strong rooted and the stem is upright, multi-branched, 30-120 cm high, spiny glands and white or brown hairs [11]. The results of biological studies have revealed that licorice extract exhibits antioxidant, anti-inflammatory, antiviral, cytotoxic, anti-diabetic activities and it is widely used to treat hepatitis, bronchitis, and malaria [13-15]. The main pharmacological components in the root of *G. uralensis* are glycyrrhizic acid and liquiritin [14,15]. Although numerous studies have been conducted to explore the application of *G. uralensis* extracts for medicinal purposes [16], its antagonistic effects against the nematode *Meloidogyne incognita* remain to be studied. Therefore, the current study aimed to evaluate the nematicidal activity of *G. uralensis* root extract (GuRE) against *M. incognita* and determine the major chemical constituents responsible for its bioactivity.

Materials and Methods

Plant materials

The roots of *G. uralensis* were procured from Sapa (Lao Cai, Vietnam). The root extract was generated following the method described by Nguyen et al. [17], with minor modifications. The dried *G. uralensis* root sample (1 kg) was extracted with methanol (80%, 3 times) for 24 h.

MeOH extracts were filtered, evaporated to dryness under vacuum at 40 ºC (N-1000; Eyela, Tokyo, Japan), and lyophilized to obtain GuRE. The GuRE recovery yield accounted for 22.47% of the initial dry weight of the plant material, and the GuRE was refrigerated until use.

Collection of eggs and *M. incognita* second-stage juveniles (J2)

Meloidogyne incognita was kindly supplied by the College of Agriculture and Life Sciences, Chonnam National University (Gwangju, Republic of Korea). *Meloidogyne incognita* was collected from the roots of infected tomato plants. Briefly, the galled root samples were washed with water, cut into 4–8 mm long pieces, and transferred into a conical flask containing a mixture of distilled water and NaOCl [18,19]. The eggs and juveniles of *M. incognita* were collected using a modified Baermann funnel method [20-22].

Hatch inhibition assay

GuRE dissolved in methanol at concentrations of 0, 0.25, 0.5, 1.0, and 2.0 mg/mL was employed to immerse approximately 500 egg suspensions of the mixed-development stage in a 24-well Microtest tissue culture plate (Becton, Dickinson and Company, Franklin Lakes, NJ). The plates were sealed with parafilm to prevent evaporation and were kept in a humid chamber at 26±2 ºC. The number of eggs and J2s of *M. incognita* were counted at 0, 5, and 10 d (D0, D5, and D10, respectively) after treatment (Leica DM2500 LED; Leica Microsystems CMS GmbH,Wetzlar, Germany). All the experiments were repeated twice with six replicates. The following formula was used to calculate the cumulative percentage of egg hatch inhibition [23]:

$$\text{Hatch inhibition (\%)} = \frac{\text{no. of J2 at D5/D10} - \text{no. of J2 at D0}}{\text{Egg at D0}} \times 100 \quad (1)$$

Mortality assay

GuRE was dissolved in methanol at a concentration of 0, 0.25, 0.5, 1.0, and 2.0 mg/mL and used for toxicity testing against J2s of *M. incognita*; 1% methanol was used as the negative control. At 0, 6, 12, 24, and 48 h after treatment, 20 µL of nematode suspension was taken out using pipette and the number of alive and dead nematodes was determined using a microscope, and nematodes were considered dead when they were completely motionless after being poked with a needle. The mortality of the J2s was evaluated after 0, 6, 12, 24, and 48 h of treatment and then calculated according to the following formula [24]:

$$\text{Relative mortality (\%)} = \frac{\text{mortality percent of treatment} - \text{mortality percent of untreated control}}{100 - \text{mortality percent of untreated control}} \quad (2)$$

The experiments were performed twice, with three replicates for each treatment.

Statistical analysis

The repeated measure analysis of variance (ANOVA) with SAS University Edition (SAS Institute Inc., Cary, NC, USA) was used to analyze the data from the hatch inhibition and mortality analysis assays. Probability levels of $p \leq 0.05$ were considered statistically significant.

Results and Discussion

GuRE-induced *M. incognita* hatch inhibition

Substantial advances have been made recently to use plant extracts for PPN management with varying efficacies. The effects of plant extracts from different parts, such as roots, barks, leaves, seeds, and fruits, have been evaluated on various important PPNs in crop production [25]. To date, over 348 plant species belonging to approximately 81 families have been screened for nematicidal bioactivity [10]. Studies on these botanical materials highlight the importance of identifying a bio-nematicide for the most destructive PPN species. Considering this, *Meloidogyne* spp. stand out as one of the main targets of bio-nematicides [10].

To the best of our knowledge, this is the first report on the effect of *G. uralensis* extracts on nematodes *in vitro*. Maestrini et al. [26]

demonstrates toxicity of licorice extract against *Trichostrongylus* and *Teladorsagia*, and that *G. glabra* extracts exhibited activity against RKN [27]. The use of medicinal plant extracts against PPNs has increased in recent years because of their low cost and little or no side effects [2,19,28]. In this study, the organic and aqueous extracts used for hatch inhibition assays against *M. incognita* exhibited significant activity (Table 1). Hatch inhibition varied based on the extracts and concentrations used for the assay. The results revealed that, at 5 d after treatment (DAT), the hatch inhibition was 21.3, 38.7, 56.0, and 64.0% at 0.25, 0.5, 1.0, and 2.0 mg/mL GuRE, respectively. The corresponding values at 10 DAT were 21.4, 36.1, 62.7, and 68.1%, respectively (Table 1). In contrast, hatch inhibition was 6.7 and 2.7% after treatment with MeOH control for 5 and 10 d, respectively (Table 1). The LC_{50} and LC_{90} values of GuRE for hatch inhibition of *M. incognita* at 5 DAT were 1.21 and 2.67 mg/mL, respectively. The corresponding values at 10 DAT were 1.13 and 2.39 mg/mL, respectively (Table 1).

Table 1 *Meloidogyne incognita* hatch inhibition (%) using different concentrations (0, 0.25, 0.5, 1.0, and 2.0 mg/mL) of *G. uralensis* root extract (GuRE) for 5 and 10 d

Concentration of GuRE (mg/mL)	Hatch inhibition (%)	
	5 d	10 d
MeOH	6.7±1.9^{e}	2.7±1.4^{e}
0.25	21.3±2.1^{d}	21.4±3.6^{d}
0.5	38.7±2.1^{c}	36.1±2.5^{c}
1.0	56.0±3.6^{b}	62.7±2.6^{b}
2.0	64.0±3.2^{a}	68.1±2.7^{a}
LC_{50} (mg/mL)	1.21	1.13
LC_{90} (mg/mL)	2.67	2.39

Each value represents the mean ± standard deviation. Means followed by different letters in a column are significantly different based on Tukey's test ($p \leq 0.05$)

Lethal effects of GuRE on the relative mortality of *M. incognita* juveniles

The nematicidal activity of GuRE on the J2s of *M. incognita* was tested. GuRE was more effective at causing the mortality of *M. incognita*, as shown in Table 2. The relative mortality of J2 after treatment with MeOH control and 2.0 mg/mL GuRE for 48 h was 2.83 and 96.24%, respectively (Table 2). When 1.0 mg/mL GuRE was used, the relative mortality of J2 was 50.50, 73.09, and 85.54% after treatment for 6, 12, and 24 h, respectively. The corresponding values for 2.0 mg/mL GuRE were 76.67, 93.07, and 97.14%, respectively (Table 2). The LC_{50} values of GuRE for the relative mortality of *M. incognita* J2s after treatment for 6, 12, 24, and 48 h were 1.14, 0.60, 0.42, and 2.67 mg/mL, respectively. The corresponding values for LC_{90} were 2.22, 1.66, 1.46, and 1.39 mg/mL, respectively (Table 2).

Our results are consistent with those of previous studies where the nematicidal effects of plant extracts in methanol were demonstrated [15,19,29]. Various *G. uralensis* extracts have been reported to have antibacterial, antifungal, and antiviral properties. For instance, a crude alcoholic root extract of *G. uralensis* or its purified compounds showed bioactivities against human pathogens such as *Arthrinium sacchari*, *Chaetonmium funicola*, and *Candida albicans* [30-32]. In our study, the relative mortality rate of *M. incognita* J2s increased with increasing GuRE concentration, indicating that it was dependent on both the concentration and treatment time (Table 1; Table 2). These results are consistent with those of previous studies where medicinal plant extracts were shown to change the morphology of *M. incognita* J2s and eggs [15,33,34].

Effects of GuRE on the morphology of *M. incognita* eggs and juveniles

In this study, the changes in egg morphology were determined at 5 DAT (Fig. 1). Eggs appeared abnormal and deformed at GuRE concentrations of 1.0 and 2.0 mg/mL. Interestingly, eggs appeared nonuniform and degraded at 5 DAT with 2.0 mg/mL GuRE. In contrast, the eggs hatched into juveniles at 5 DAT in the control (Fig. 1). Furthermore, the J2 nematodes were not only dead but also showed a broken morphology (Fig. 2) after treatment with 1.0 and 2.0 mg/mL GuRE for 48 h; however, no changes were

Table 2 The relative mortality of *M. incognita* juveniles treated with different concentrations of GuRE for 6, 12, 24, and 48 h

Concentration of GuRE (mg/mL)	J2 relative mortality (%)			
	6 h	12 h	24 h	48 h
MeOH	1.91±1.48^{e}	2.93±0.24^{d}	1.96±1.52^{e}	2.83±2.55^{d}
DW	0^{e}	1.92±1.50^{d}	1.01±1.56^{e}	0.93±1.44^{d}
0.25	14.01±2.35^{d}	44.84±4.00^{c}	53.37±1.43^{d}	60.93±3.09^{c}
0.5	37.45±4.18^{c}	65.15±1.61^{b}	76.74±4.15^{c}	78.11±3.74^{b}
1.0	50.50±2.64^{b}	73.09±4.35^{b}	85.54±4.16^{b}	94.28±0.14^{a}
2.0	76.67±3.40^{a}	93.07±2.96^{a}	97.14±2.55^{a}	96.24±3.78^{a}
LC_{50} (mg/mL)	1.14	0.60	0.42	0.32
LC_{90} (mg/mL)	2.22	1.66	1.46	1.39

Each value represents the mean ± standard deviation. Means followed by different letters in a column are significantly different based on Tukey's test ($p \leq 0.05$)

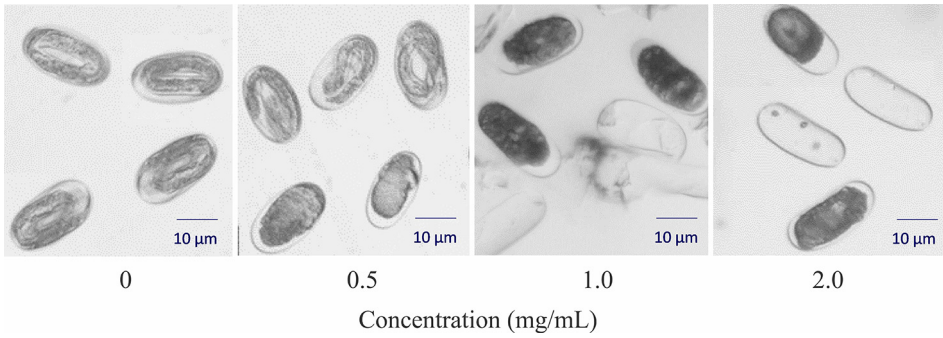

Fig. 1 Light microscopy images of *M. incognita* eggs after treatment with GuRE (0, 0.5, 1.0, and 2.0 mg/mL) for 5 d

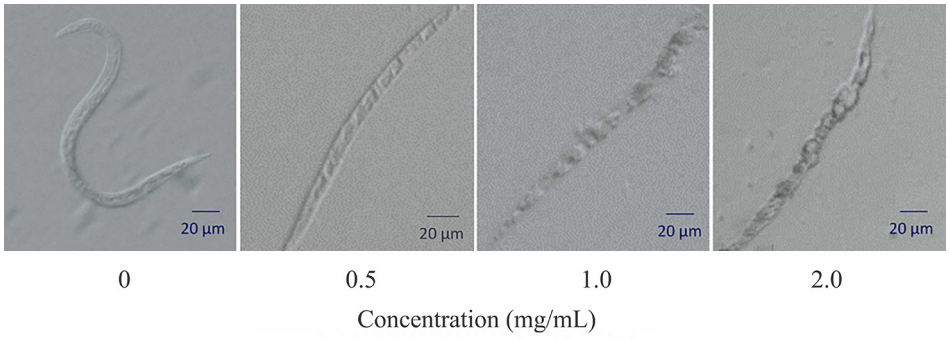

Fig. 2 Light microscopy images of *M. incognita* J2s after treatment with GuRE (0, 0.5, 1.0, and 2.0 mg/mL) for 48 h

observed in the control (Fig. 2).

The presence of chitin has been documented in a variety of different nematode species; it is a common structural component of the nematode eggshell [35-37]. An eggshell surrounds the eggs, and a chitinous layer provides strength [38]. Blocking the synthesis of chitin is one of the mechanisms through which different compounds break the fungal cell wall and exert their antifungal activity [39]. OuYang et al. [40] reported that the cell wall of *Geotrichum candidum var. citri-aurantii* was dissolved and the chitin content was reduced after treatment with cinnamaldehyde; thus, it inhibits mycelial growth by destroying the integrity of the fungal cell wall. The GuRE recovery yield accounted for 22.47% of the dried *G. uralensis* Fisch. roots (dried weight), and GuRE was yellow in color (Data not shown). The components from *G. uralensis* possess antiulcer, anti-inflammatory, anti-allergic, antithrombotic, antidiabetic, hepatogenic, and neuroprotective activities [13,41].

Further studies are required to investigate the efficacy of GuRE against plant nematodes in soil and plant assays. In addition, purifying the active compounds from GuRE is imperative to ascertain their specific role against RKNs. The current study suggests that GuRE could be used as a safe and environment-friendly bio-nematicide against *M. incognita*.

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Declarations

Competing Interests No potential conflict of interest was reported by the author(s).

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