Identification of Two Parasitic Nematodes in The Rhizosphere of *Centranthera grandiflora* in Pingbian, Yunnan

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Abstract. *Centranthera grandiflora*, a kind of traditional Chinese medicinal materials in Yunnan Province, has very high medicinal value. However, constant expansion of the plantation has resulted in worsening root diseases, an obstacle of continuous cropping. From 2018 to 2019, the author investigated the parasitic nematodes in the rhizosphere of *Centranthera grandiflora* in Pingbian County, Yunnan province, and identified them according to their morphological characteristics. The results showed that there were two kinds of parasitic nematodes harmful to *Centranthera grandiflora* root in Pingbian County, Yunnan: *Tylenchorhynchus capitatus* Allen, 1955 and *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961.

1 Introduction

Centranthera grandiflora, also known as smooth wild bean, Small health potion, etc., is the whole grass of *Centranthera grandiflora* under the Scrophulariaceae category [1]. People call it "Plants gold in the mountains", and it is mainly grown in Guangxi, Guizhou, Yunnan and other places [2]. *Centranthera grandiflora* has anti-myocardial ischemia and anticoagulant effect [3]. It has significant effects of promoting blood circulation and removing blood stasis, providing energy and enriching blood, as well as curative effects of external bleeding, internal bleeding and gastroenteritis (Yunnan Honghe Chinese Herbal Medicine Manual). *Centranthera grandiflora* in Yunnan is of excellent quality, making it popular in both domestic and international markets. It is currently grown in Wenshan, Honghe and other areas. It's a rare Chinese medicine in the local area with a huge demand. Due to the expansion of plantation, more diseases and pests are emerging, which seriously affected the quality of *Centranthera grandiflora*, especially nematode affecting the root. Plant parasitic nematodes can transmit viruses, fungi and bacteria to form complex diseases [4]. *Tylenchorhynchus* and *Helicotylenchus* are important plant pathogen nematodes, which can harm plant roots and cause poor growth of the aboveground. Parasitic nematodes have become one of the important factors impeding the growth of *Centranthera grandiflora*. So far, there are few studies on parasitic nematodes of *Centranthera grandiflora*. Therefore, the author investigated and identified the species of parasitic nematodes affecting *Centranthera grandiflora* in Pingbian county, Yunnan province during 2018 and 2019, in order to provide references for the

diagnosis and control of root diseases of *Centranthera grandiflora*.

2 Materials and Methods

2.1 Collection of rhizosphere soil of *Centranthera grandiflora*

The 10 samples of rhizosphere soil collected from the planting area of *Centranthera grandiflora* in Pingbian County in 2019 were studied based on key symptoms including weak growth of aboveground, poor growth, wilting, browning or swollen and deformed roots. Rhizosphere soil samples were collected by "quartering" method and put into the marked self-sealing bag, which was brought back to the lab and separated nematodes timely.

2.2 Collection of rhizosphere soil of *Centranthera grandiflora*

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3 Results and Analysis

Two species of parasitic nematodes were isolated and identified from soil samples collected from the planting area of *Centranthera grandiflora* in Pingbian, Yunnan. The first species was *Tylenchorhynchus capitatus* Allen, 1955 that is classified as *Tylenchorhynchus* in the subfamily of *Tylenchorhynchidae* under *Tylenchida*. The second species was *Helicotylenchus dihystra* (Cobb,1893) sher, 1961, classified as *Helicotylenchus* in the *Hoplolaiminae* family under *Hoplolaimida*.

3.1 *Tylenchorhynchus capitatus*

Morphological features: the body is ventrally curved after the fixation, which is 582-657 μm long and 20-23 μm wide with fine lines. The excretion hole is usually located in the front half of the esophagus gland. The meniscus is not obvious, the lip area round, protruding, and the base being over-contracted. It has a strong stylet, the base of which is round. The middle esophagus bulb is ovoid, the isthmus is narrow, the esophagus gland is long and pear-shaped, which does not cover the intestine, has a clear boundary with intestine, and the cardia is obvious. The vulva is usually concave in the middle of the body, and the two ovaries feature straight extension with single or multiple lines of oocytes. The conical tail is ventral curved and tapering, with the tail being 38.9-47.0 μm in length. The tail has fine, regular or irregular ring pattern. The heart-shaped tail end has no ring pattern, and protrudes in the body ring. The morphological characteristics are shown in Figure 1, and the comparison of measured values and literature records are shown in Table 1.

Figure 1. *Tylenchorhynchus capitatus*
 A. Female anterior; B. Female vulva; C. Female tail

3.2 *Helicotylenchus dihystra*

Morphological features: After killed in the water bath, the female body was spirally shaped, with clear ring lines, posterior esophageal gland covering the intestine with a long ventral cover. Some female body only circled around the rear half of the body for 1 circle, while some of the front part was almost straight, and the rear part almost rotated for 3 circles. The head is semispherical, or slightly flat in the front end. It has 4-5 invisible rings, and the front of the base ball of the stylet

feature flat to dentate shape. The excretory hole is located before the bowel valve of esophagus, the labial of vulva is slightly sunken with two gonads facing each other. Oocytes are arranged in a single row, with round oocyst that has no sperm inside. The conical tail is ventral curved and asymmetric cone, the ventral end of the tail has a prominent protuberance.

The morphological characteristics are shown in Figure 2, and the comparison of measured values of *Helicotylenchus dihystra* and those in the literature are shown in Table 2.

Figure2. *Helicotylenchus dihystra*
 A. Female anterior; B. Female vulva; C. Female tail

4 Discussion

The results showed that there were two parasitic nematodes in the rhizosphere of *Centranthera grandiflora* in Pinbian county Yunnan province: one was *Tylenchorhynchus capitatus* Allen, 1955; the other was the *Helicotylenchus dihystra* (Cobb,1893) sher, 1961. The morphology characteristics of *Tylenchorhynchus capitatus* is basically consistent with descriptions by Feng Zhixin [7]. In the morphological index measurments, a (length/width) is slightly smaller than the literature value, and the stylet is slightly longer than the literature value, while the rest of the indicators are consistent with the literature records. This is probably due to geographical differences between populations. Therefore, it was identified as *Tylenchorhynchus capitatus*. The morphological characteristics of the *Helicotylenchus dihystra* were consistent with descriptions by Feng Zhixin in 1982, and the morphological indicators were basically consistent with the values in the literature [7], so it was identified as the *Helicotylenchus dihystra*. The damages of these two nematodes to *Centranthera grandiflora* need further study.

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Table 1 The Measured Values of *Tylenchorhynchus capitatus* of *Centranthera grandiflora* and those reported in the literature

Morphological index	Group of Mengzi	Literature ^[7]
n	12♀♀	10♀♀
L (μm)	617.0±53.0 (581.7~657.0)	(580~630)
W (μm)	21.5±0.4 (20.4~22.7)	(21~27)
Tail (μm)	42.5±5.4 (38.9~47.0)	(37~53)
a	27.8±0.4 (25.4~29.9)	(30~38)
b	5.0±0.1 (4.9~5.1)	(5.0~5.8)
c	14.5±0.4 (14.0~15.1)	(12~17)
A-vulva	338.2±55.8 (299.0~376.0)	—
V	54.7±4.1 (51.3~57.9)	(51~58)
ST	17.2±0.9 (16.8~17.9)	17
DGO	3.7±0.9 (2.1~4.6)	(2.0~4.0)
Ex.p	132.0±13.9 (122.7~143.8)	(108~137)
Oes	145.8±11.9 (138.4~155.1)	(138~158)
Bulbl	19.1±2.0 (16.4~21.2)	(15~20)
bulbw	11.7±1.3 (10.7~12.8)	(10~12)

Table 2 The Measured Values of *Helicotylenchus dihystra* of *Centranthera Grandiflora* and those reported in the literature

Morphological index	Group of Mengzi	Literature ^[7]
n	13♀♀	10♀♀
L(μm)	709.8±78.7(624.8~930.0)	(620~840)
W(μm)	25.2±3.2(21.3~32.9)	(20.5~27.5)
AW(μm)	16.2±2.5 (12.0~21.3)	—
Tail(μm)	17.6±2.6(14.4~24.63)	(10.5~24.5)
a	28.3±1.2(26.0~30.2)	(21~32)
b	5.0±0.2(4.6~5.3)	(4.4~6.1)
c	40.7±2.9(35.4~43.9)	(35~49)
c'	1.1±0.1 (1~1.2)	(0.8~1.2)
ST	25.1±1.6(23.7~26.4)	(24~29)
A-vulva	454.0±49.7 (398.8~593.1)	—
V	64.0±1.5 (60.0~66.2)	(60.6~65.9)
DGO	10.2±1.1 (8.3~11.8)	(8~11.2)

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