

## Genistein and Daidzein effects on the physiological indices of Soybean Cyst Nematodes

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**ABSTRACT:** Soybean cyst nematode (SCN, *Heterodera glycines*) is a chief plant–parasitic nematode of soybean. Application of synthetic chemical nematicides poses negative side effects to human health and the environment. Therefore, the search for a safe and effective approach is more relevant. This study evaluated the effects of Genistein and Daidzein on the physiological index of soybean cyst nematodes, individual morphology, reversal frequency, respiration, and body fluid leaking of second–stage juveniles (J2s) of soybean cyst nematode. The results showed that body length of J2s decreased, while stylet and tail transparent area elongated. Additionally, after the treatment, the body became hollow and shrunken, the J2s stiffened, whereas the reversal frequency decreased dramatically after 24 h of treatment. Moreover, the body fluid leakage was intensified and respiration was inhibited. Oxygen consumption decreased by 86.7 % and 70.1 %, while, in contrast, electrical conductivity increased by 40.1 % and 36 % at 100 µg mL<sup>-1</sup> of Genistein and Daidzein, respectively, after 48 h of exposure. The smaller number of J2s in soybean roots, the slower development rate, and the abnormal sexual differentiation were found in greenhouse assay. Thus, Genistein and Daidzein, especially Genistein, have strong effects on the physiological index of soybean cyst nematodes. Moreover, the effects were time and dosage–dependent.

**Keywords:** *Heterodera glycines*, nematicides, respiration, morphology

### Introduction

Nematoda is the phylum of Kingdom Animalia that can be found in almost any type of environment (Blaxter and Denver, 2012). With long–term evolution, nematodes have developed specialized morphological features, cuticles, muscles, nervous and digestive systems suitable for the habitats and feeding habits. An example is the mouthpart of plant parasite nematode, a needle–like stylet, which is used to puncture cells during feeding (Yeates et al., 1993). Stylet activity is a necessary part for invasion of and migration through roots (Bell et al., 2019). Stylet activity can be affected by chemical or bio–factors, such as exposure to root exudate, and is known to stimulate stylet thrusting in some plant–parasitic nematodes (Warnock et al., 2016).

Soybean cyst nematode (SCN, *Heterodera glycines*) is one of the most devastating diseases in soybean crops worldwide. Soybean yield loss due to *H. glycines* has been estimated to amount more than US\$ 1.5 billion worldwide (Hosseini and Matthews, 2014). Once SCN penetrates and feeds inside the root tissue, juveniles sedentary endoparasites remain in a permanent feeding site inside the root for their remaining life (Yeates et al., 1993). The only state that infects soybean roots of SCN is J2. Subsequently, nematodes develop into males or females throughout three molts (J3, J4, and adults). Therefore, chemicals that affect the activity of J2, poisoned them, reduce J2 infestation ability, or even kill them, inhibiting their infection to plant roots and reducing the damage of SCN to soybean.

Secondary metabolites have high antioxidants and nematicidal ability and they cause deterioration to internal tissues and cuticle of nematodes (Lai et al., 2014). Other

studies have demonstrated that isoflavone has a repelling effect on *Caenorhabditis elegans* and *Bursaphelenchus xylophilus*, affecting their movement and hatching (Lee et al., 2008; Zhao et al., 2007). Evidence shows that isoflavonoids impede SCN replication by influencing the sex ratio and the number of female eggs (Chin et al., 2018). Although studies have shown that exogenous isoflavones have the positive suppression ability on SCN J2s (Guo et al., 2017), their specific mode of action on SCN J2s is still unclear. Therefore, this study assessed the effects of Genistein and Daidzein on the morphology, oxygen consumption, body fluid infiltration, and reversal frequency of SCN J2s to better understand the physiological effects of Genistein and Daidzein on nematodes.

### Materials and Methods

#### Nematode collection method

SCN race 3 was used in this study. Cysts were obtained from the experimental field in Shenyang, Liaoning, China (41°50'4" N, 116°19'53" E, altitude 70 m) and collected by following elutriation and hand–picking under a stereomicroscope. Cysts were surface–sterilized by immersion in 0.1 % HgCl<sub>2</sub> solution for 1 min followed by rinsing three times with sterile distilled water. The cysts were then placed in Baermann funnel at 25 °C. Second–stage juveniles were collected from the bottom of the Baermann funnel every two or three days (Liu, 1995).

#### Preparation of Genistein and Daidzein solution

Genistein and Daidzein purchased from Merck KGaA, Darmstadt, Germany, were dissolved to 10 µg mL<sup>-1</sup>, 50

$\mu\text{g mL}^{-1}$ , and  $100 \mu\text{g mL}^{-1}$  concentrations in dimethyl sulfoxide (DMSO, final volume less than 1 %), respectively, and frozen in aliquots until ready for use.

### Individual morphological assay

SCN J2s were treated with different concentrations of Genistein and Daidzein under room temperature for 24 h, and then body length, stylet, and tail transparent area of J2s were measured under the microscope. Sterile water with a small amount of DMSO (final volume of less than 1 %) was used as the control. Three independent experiments were conducted and each experiment included three replications.

### Reversal frequency assay

Fresh and active SCN J2s were placed in a Petri dish filled with 1 mL Genistein and Daidzein. The movement behavior was measured according to the method of Tsalik with some modifications (Tsalik and Hobert, 2003). All assay dishes were pre-equilibrated to the room temperature. The reversal frequency (RF) of J2s was measured at 1, 3, 6, 12, and 24 h, with 1 min for each hour under stereomicroscope. Any changes from forward to the backward movement were scored as a reversal. Three replicates were set for each treatment and the nematodes treated with sterile water with a small amount of DMSO were used as the control.

### Respiration (oxygen consumption) assay

Respiration of SCN J2s was measured by the oxygen electrode method to determine the effect of isoflavone on respiration of SCN J2s (Kohra et al., 2002; Qi et al., 2008). Approximately 20,000 SCN J2s were added into the solution that contained 10, 50, or  $100 \mu\text{g mL}^{-1}$  of Genistein or Daidzein, respectively. Nematode suspension with a small amount of DMSO was used as the control. Oxygen consumption of J2s was measured by JPSJ-605 dissolved oxygen meter at 1, 3, 6, 12, 24, and 48 h in each treatment group. Three replicates were set for each treatment. The test was conducted in a water bath at  $25 \text{ }^\circ\text{C}$ . The control solution in the formula was the isoflavone solution with corresponding concentration and sterile water.

$O.C \text{ of SCN J2s} = \text{oxygen in control solution} - \text{oxygen in SCN J2s suspension}$

where: *O.C.* is Oxygen Consumption of SCN ( $\text{mg L}^{-1}$ ).

### Body fluid infiltration assay

Fluid leakage of J2s was studied by the electrical conductivity method (Kohno et al., 1981). Approximately 20,000 SCN J2s were added into the solution that contained 10, 50 or  $100 \mu\text{g mL}^{-1}$  of Genistein or Daidzein, respectively. Nematode suspension with a small amount

of DMSO was used as the control. DdSJ-308a conductivity meter was used to determine the conductivity of SCN J2s at 1, 3, 6, 12, 24, and 48 h in each treatment group. Three replicates were set for each treatment. In the formula, the control solution comprises an isoflavone solution and sterile water with corresponding concentration.

$E.C \text{ of SCN J2s} = E.C. \text{ of SCN J2s suspension} - E.C. \text{ control solution}$

where: *E.C.* is Electric Conductivity of SCN ( $\mu\text{S cm}^{-1}$ ).

### Greenhouse experiment (Infectivity and development assay)

The experiment was carried out in a greenhouse of Shenyang Agricultural University, China ( $41^\circ49'33'' \text{ N}$ ,  $134^\circ34'9'' \text{ E}$ , at 72 m a.s.l.). The Liaodou15 soybean cultivars susceptible to SCN were used. The seeds were surface-sterilized with 0.5 % NaClO for 10 min and washed several times with sterile distilled water, air-dried, and then germinated on filter paper in distilled water in Petri dishes and incubated at  $28 \pm 1 \text{ }^\circ\text{C}$  for one week. The seeds were sown in 18-cm plastic pots that contained a sterile soil mixture (topsoil: sand: vermiculite, 3:2:1). The seedlings were inoculated with 2,000 living J2s treated with different concentrations of Genistein and Daidzein at the cotyledon stage for 24 h. J2s treated with sterilized water were used in the control. Three days after inoculation, soybean seedlings were transferred to new plastic containers filled with sterile soil mixture and irrigated with 1/4 Hoagland nutrient solution in a growth chamber under normal conditions ( $25/20 \text{ }^\circ\text{C}$  day/night temperature, relative humidity 60–80, and 16 h light period/day at the intensity of  $160 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ). The development rate was observed accurately. To avoid further penetration of J2s, seedlings were moved to new containers. The number of females of J2s, J3s, and J4s inside the roots at 7 dpi, 14 dpi, 21 dpi, and 28 dpi were observed and counted using a stereomicroscope after the roots were stained with acid fuchsin (Hussey, 1985; Sikandar et al., 2019). The experiment was repeated three times.

### Statistical Analysis

The SPSS17.0 software was used to analyze the significance of the difference under the conditions of the Duncan's multiple range test  $p < 0.05$  and Graphpad Prism v.8.0. were used to analyzed the experimental data and draw the charts.

## Results

### Individual morphology assay

Both Genistein and Daidzein affected the body length, stylet, and tail translucency of SCN J2s. Genistein had

a more pronounced effect on SCN J2s compared to Daidzein, and this effect deepened with an increase in the concentration of isoflavone. The body length of SCN J2s was treated with 100  $\mu\text{g mL}^{-1}$  and 50  $\mu\text{g mL}^{-1}$ . Genistein was greatly reduced, followed by 100  $\mu\text{g mL}^{-1}$  Daidzein-treated nematodes. In terms of stylets and tail translucent region, with the exception of 10  $\mu\text{g mL}^{-1}$  Daidzein, the other treatment was considerably higher. The influence of Genistein on the stylet and the transparent tail area was slightly higher than that of Daidzein at the corresponding concentration (Table 1).

### Reversal frequency assay

Nematodes displayed a non-directional thrashing behavior in all treatments; however, the reversal

frequency of SCN J2s slowed down to a certain extent in both isoflavones treatments. The reversal frequency of SCN J2s decreased correspondingly with the increasing time and isoflavone concentration. Genistein, at 100 and 50  $\mu\text{g mL}^{-1}$  concentrations, imposed a greater effect on the reversal frequency. The nematodes swung much slower in Genistein than in Daidzein at 12 h after treatment and most nematodes were in a rigid state after 24 h. Daidzein, at 10  $\mu\text{g mL}^{-1}$  concentration, had little effect on the nematode swing frequency in the early stage and even promoted its swing phenomenon after treatment for 1 h. Daidzein had a relatively large effect on the swing frequency at a concentration of 100  $\mu\text{g mL}^{-1}$  after treated 24 h. Some nematodes waved slowly, even basically lost their ability to move or died (Figure 1).

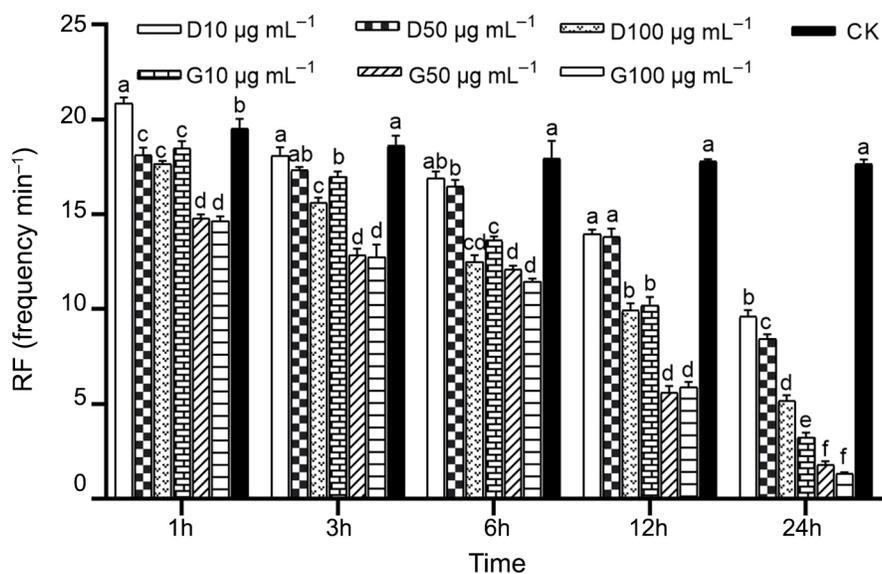
### Respiration (oxygen consumption) assay

Genistein affected the respiration of SCN J2s. Compared with the control, oxygen consumption of nematodes treated by Genistein was significantly reduced after 6 h of treatment. Oxygen consumption of nematode treated by Genistein at 100  $\mu\text{g mL}^{-1}$  and 50  $\mu\text{g mL}^{-1}$  showed a sharp decrease and almost stopped after 48 h. The effect of 100  $\mu\text{g mL}^{-1}$  Daidzein was similar to that of 100  $\mu\text{g mL}^{-1}$  Genistein. Oxygen consumption in 12–48 h decreased linearly and gradually approached 100  $\mu\text{g mL}^{-1}$  Genistein. After 12 h of treatment with 100  $\mu\text{g mL}^{-1}$  and 50  $\mu\text{g mL}^{-1}$  Daidzein, the decrease of nematode oxygen consumption slowed down and tended to be flat, which was significantly different from other treatments (Figure 2).

**Table 1** – Treatment effect on the morphology of Soybean Cyst Nematode

Treat.	Body length	Stylet	Tail's transparent
D10 $\mu\text{g mL}^{-1}$	720.04 $\pm$ 2.20 <sup>b</sup>	43.39 $\pm$ 0.11 <sup>d</sup>	46.97 $\pm$ 0.39 <sup>d</sup>
D50 $\mu\text{g mL}^{-1}$	715.55 $\pm$ 2.74 <sup>c</sup>	43.74 $\pm$ 0.11 <sup>c</sup>	48.32 $\pm$ 0.45 <sup>c</sup>
D100 $\mu\text{g mL}^{-1}$	708.84 $\pm$ 2.04 <sup>d</sup>	44.10 $\pm$ 0.07 <sup>b</sup>	48.61 $\pm$ 0.36 <sup>c</sup>
G10 $\mu\text{g mL}^{-1}$	716.32 $\pm$ 1.39 <sup>c</sup>	44.08 $\pm$ 0.07 <sup>bc</sup>	49.72 $\pm$ 0.09 <sup>b</sup>
G50 $\mu\text{g mL}^{-1}$	699.35 $\pm$ 1.11 <sup>e</sup>	44.55 $\pm$ 0.47 <sup>a</sup>	51.60 $\pm$ 0.32 <sup>a</sup>
G100 $\mu\text{g mL}^{-1}$	697.53 $\pm$ 0.94 <sup>e</sup>	44.74 $\pm$ 0.05 <sup>a</sup>	51.98 $\pm$ 0.12 <sup>a</sup>
Control	725.13 $\pm$ 2.31 <sup>a</sup>	43.26 $\pm$ 0.15 <sup>d</sup>	46.70 $\pm$ 0.09 <sup>d</sup>

Data presents the mean  $\pm$  standard deviation. The same letters in columns are significantly similar according to the Duncan's multiple range test ( $p > 0.05$ ).



**Figure 1** – Reversal frequency of nematodes during Genistein and Daidzein treatments. The error bars illustrated the mean  $\pm$  standard error. Different letters on the bar indicate that values are significantly different according to the Duncan's multiple range test at  $p > 0.05$ .

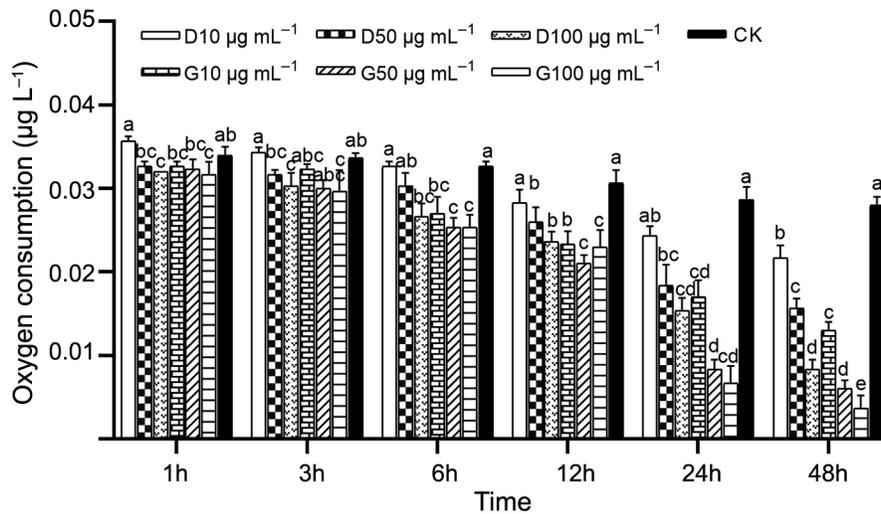
**Body fluid infiltration assay**

In the treatment of SCN J2s with two isoflavones at different concentrations, the conductivity of the nematode suspension increased steadily in the early stage of SCN J2s suspension treated with 50  $\mu\text{g mL}^{-1}$ , 100  $\mu\text{g mL}^{-1}$ , and 100  $\mu\text{g mL}^{-1}$ . The Daidzein solution rapidly increased after 6 h, while the conductivity of nematode suspension treated with 10  $\mu\text{g mL}^{-1}$  Genistein gradually stabilized. Similarly, except for conductivity at 100  $\mu\text{g mL}^{-1}$  when the Daidzein treatment was higher than that of conductivity Genistein 10  $\mu\text{g mL}^{-1}$  after 24 h, Genistein had a greater effect on nematode suspension (Figure 3).

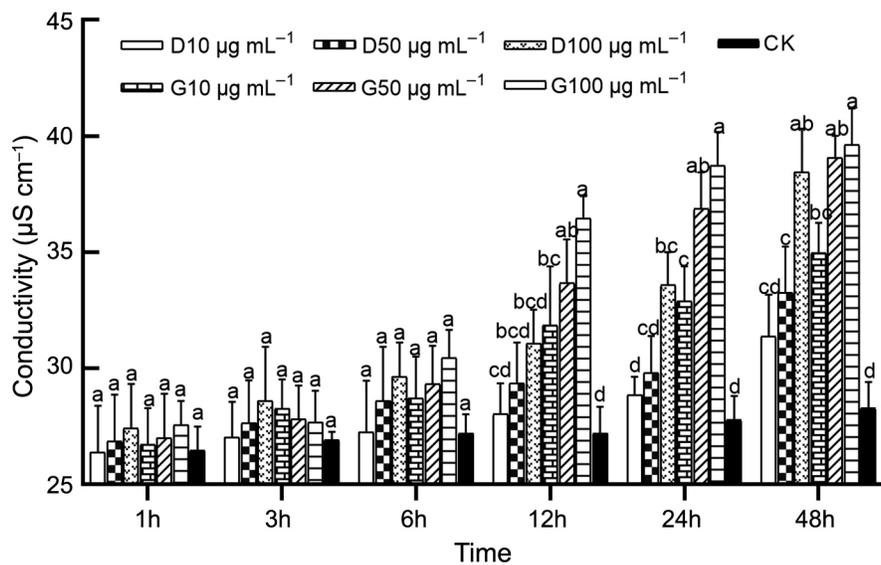
**Infectivity and development assay**

Given that two isoflavones treatments affect the body length, respiration, and other physiological indexes of J2, we decided to inoculate the treated J2s to determine whether Genistein and Daidzein treatments affected the infectivity and development of J2.

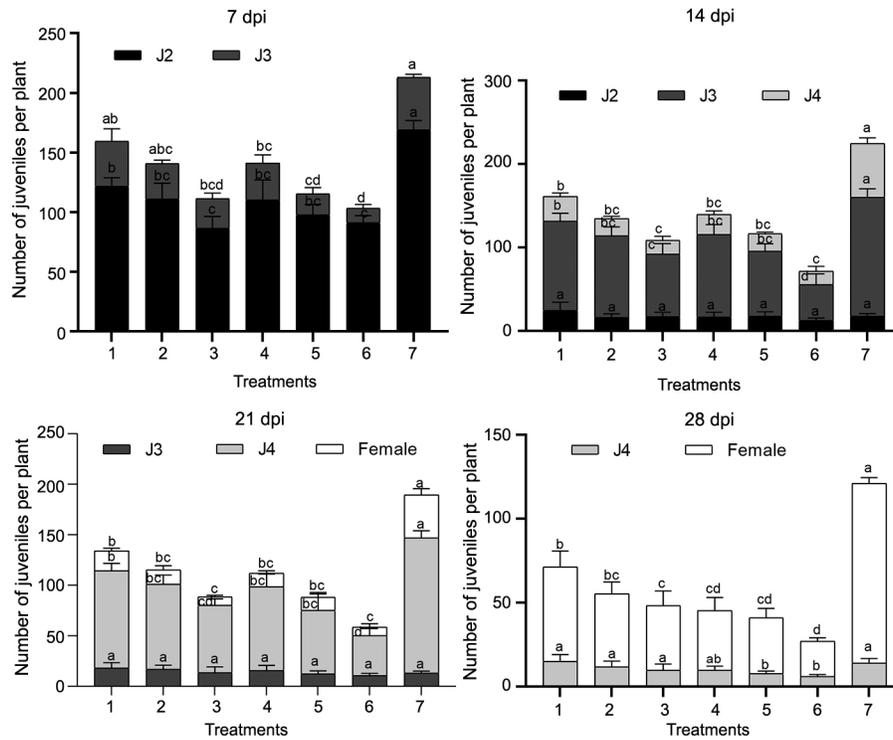
The results showed that the total number of nematodes in each treatment was significantly smaller than that of control. The number of J2s in each treatment group was significantly smaller than that of the control group at 7 dpi. As the treatment concentration increased, the number of J2s that invaded soybean root successfully was significantly reduced; however, it was



**Figure 2** – Respiration of nematodes during Genistein and Daidzein treatments. The error bars illustrated the mean  $\pm$  standard error. Different letters on the bar indicate that values are significantly different according to the Duncan’s multiple range test at  $p > 0.05$ .



**Figure 3** – Body fluid leaking during Genistein and Daidzein treatments. The error bars illustrated the mean  $\pm$  standard error. Different letters on the bar indicate that values are significantly different according to the Duncan’s multiple range test at  $p > 0.05$ .

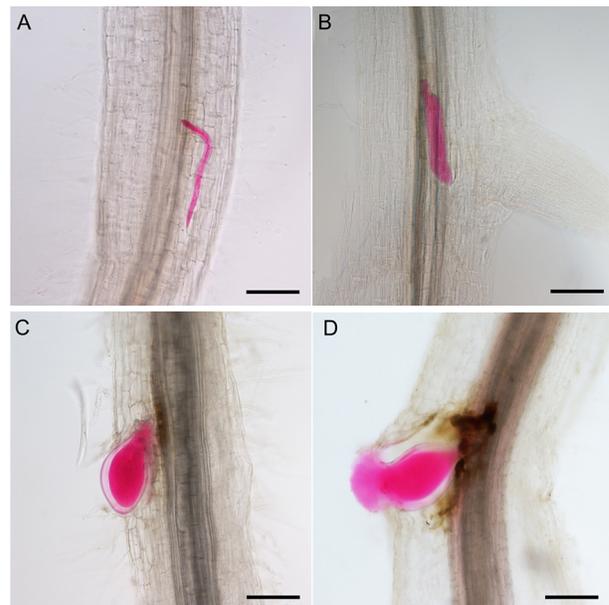


**Figure 4** – The number of Soybean Cyst Nematodes in soybean roots at different development stages after 7 dpi, 14 dpi, 21 dpi, 28 dpi inoculated with live J2s treated for 24 h with Genistein and Daidzein. The error bars illustrated the mean  $\pm$  standard error. Different letters on the bar indicate that values are significantly different according to the Duncan's multiple range test at  $p > 0.05$  (1 = D  $10 \mu\text{g mL}^{-1}$ , 2 = D50  $\mu\text{g mL}^{-1}$ , 3 = D100  $\mu\text{g mL}^{-1}$ , 4 = G10  $\mu\text{g mL}^{-1}$ , 5 = G 50  $\mu\text{g mL}^{-1}$ , 6 = G 100 D50  $\mu\text{g mL}^{-1}$ , 7 = CK).

not significantly different at the same concentration of Genistein and Daidzein. Most J2s developed into J3 at 14 dpi. At this time, the number of J2s in control was not significantly different from that in other treatments, indicating that the treatments delayed the development of J2s. J4 was the dominant state at 21 dpi. Data showed that the total number of nematodes in each treatment especially in G100  $\mu\text{g mL}^{-1}$  treatment decreased significantly, which means that J2 that successfully invaded the roots did not colonize and develop further. At 28 dpi, J4 developed into adults. Further comparison of the number of females found that isoflavone-treated J2s could affect sex differentiation during nematode development (Figures 4 and 5).

## Discussion

Admittedly, all plant-parasitic nematodes must be able to locate and feed on their host to survive. They feed on cell soluble drilled through a stylet, a thin, long, hollow, needle-like structure (Grundler et al., 1991). The nematode uses this stylet supported by muscles to puncture plant cells to secrete protein and enzyme that aids the nematode to digest plant cell wall and withdraw the cell content, such as sugar (Fanelli et al., 2014; Vieira et al., 2015), enabling plant invasion and



**Figure 5** – Development of SCN in soybean roots Bar = 200  $\mu\text{m}$ ). (A) J2 in the root system; (B) J3 in the root system; (C) J4 (♀) in the root system; (D) Female in the root system. (Photograph Credit: Yuanyuan Wang).

parasitism (Lambert and Bekal, 2002). The stylet is connected to the pharynx that, in turn, is connected to the intestine. The muscles are attached to the nematode hypodermis and surrounded the pharynx (Basyoni and Rizk, 2016), which is used to control the extension and contraction of the stylet to eject secretions from its salivary glands into and around plant cells and pump food into its intestine. Some authors reported that helminth parasite exposed to genistein caused flaccid paralysis in them and subsequent death of the parasites (Tandon and Lyndem, 2010; Toner et al., 2009), Terpenoids produce a rapid paralysis of worms and inhibit egg hatching (Hernando et al., 2019). Genistein-treated helminth parasites exhibited changes in alterations and deformity in their tegmental architecture (Kar and Tandon, 2000; Pal and Tandon, 1998). In our study, the Genistein and Daidzein treatment greatly affected the morphology of J2s. The body length of J2s shortened, while the stylet and tail transparent area elongated. The results indicated that Genistein and Daidzein cause paralysis or slack the muscles surrounding the pharynx, even the muscles of the whole body. Further, muscle paralysis or slack affected not only nematode movement and penetration, but also its feeding or withdrawal of nutrition. Many works have been carried out to clarify that nutrition is essential for nematode development and sex differentiation. Cyst nematodes are sexually dimorphic. Female cyst nematodes require, on average, 29 times more food compared with males (Muller et al., 1981). The male or female differentiation depends on host factors, such as the intensity of the host immune responses and availability of nutrients. Under favorable conditions with plenty of nutrients, more females are developed, whereas mainly male nematodes develop under nutrient deficiency (Anjam et al., 2020).

Nematodes are motile animals; nevertheless, most can move no more than a meter through the soil within their lifetime. Nematodes crawl by a wave of muscle contraction along the body in the dorsoventral plane, waves traveling from head to tail result in the forward movement. Reversals have long been recognized as a basic component of nematode locomotory behavior (Croll, 1975). A reversal frequency, previously termed an "omega turn" (Croll, 1975; Pierce-Shimomura et al., 1999), means that each animal can change from forward to backward movement within 3 min. Tsalik and Hobert (2003) demonstrated that nematode significantly decreased their reversal frequency under weakness or exhaustion due to lack of food and allowed straight movement away from unfavorable conditions. Nervous systems in nematodes vary in their complexity. Locomotory, egg-laying, defecation, feeding, intestines, and pharyngeal muscles are controlled by a small number of specialized motor neurons (Schafer, 2016). The muscles are attached longitudinally to the nematode hypodermis, allowing them to move only in a sinusoidal (snake-like) motion. Isoflavones are

capable of inducing quiescence through slowing the movement of juveniles of plant-parasitic nematodes and repelling them by changing their migration towards the roots (Chin et al., 2018). Previous studies have also demonstrated that isoflavone inhibited or slowed the motility of *Heterodera zea*, *Meloidogyne javanica* and *Meloidogyne incognita* juveniles (Faizi et al., 2011; Shaukat et al., 2003; Sommerville and Davey, 2002; Wuyts et al., 2006). Genistein and terpenoids have shown to cause paralysis and alterations in the tegument and tegumental enzymes of nematodes (Tandon and Das, 2018). Exposure to 0.5 mg mL<sup>-1</sup> Genistein provided a pronounced reduction of the activity of non-specific esterases (NSE) and cholinesterase (ChE), which were found in close association with the central and peripheral nervous components (Maule et al., 1993). The activity of acetylcholinesterase (AChE), the specific ChE in the parasite, points towards acetylcholine, an inhibitory neurotransmitter (Pal and Tandon, 1998). The reversal rate declined significantly after 24 h of treatment and the effects of treatment with Genistein and Daidzein ultimately resulted in muscle paralysis and damage to the nervous system of nematodes. Nematodes lack defined respiratory or circulatory systems (Basyoni and Rizk, 2016). Diffusion of water, gasses, and metabolites were depended on their semi-permeable body walls and internal transport. Oxygen consumption of nematodes is 50 times more than that of humans (Duan et al., 2011). Recent evidence underlines mitochondrial function as a potential contributor to the maintenance of organismal homeostasis and viability (Vafai and Mootha, 2012). The rate of oxygen consumption is a vital marker indicating cellular function under normal or metabolically challenged conditions, including a fundamental indicator of mitochondrial function, reflecting on reactive oxygen species (ROS) production and metabolic activity during lifetime under normal or metabolically challenged conditions. Preez developed a novel method to measure the oxygen consumption rate of *C. elegans* and showed that the oxygen consumption rate is used as a promising functional measurement of toxicity (Preez et al., 2020). Respiration of *C. elegans* has also been used, although infrequently, to study the effect of toxicant exposure (Schouest et al., 2009). Isoflavones alter the mitochondrial respiration, as a result oxygen consumption of *M. javanica*, which is reduced and plays a vital role in the resistance of nematodes (Cesco et al., 2012). Our results also revealed that respiration inhibition was directly related to the Genistein and Daidzein concentration and exposure time. Coincidentally, the damage of cuticle in the body fluid infiltration assay also decreased the respiration rate of nematodes.

All nematodes have an external structure known as the cuticle, which is crucial for their development and survival (Page et al., 2014). The muscle layer and the cuticle enclose a fluid-like cavity, which is filled with fluid and hemolymph that appears as a clear,

pink, almost cell-free solution that provides pressure against the cuticle to keep body shape, allowing movement (Basyonu and Rizk, 2016). Previous studies have reported that secondary metabolites or proteases from nematophagous bacteria and fungi, such as *Bacillus* spp., *Hirsutella rhossiliensis*, *Paecilomyces lilocinas* and *Pezizomycotina* spp. showed strong nematicidal potential and caused degradation to internal tissues and cuticle of nematodes (Lai et al., 2014; Son et al., 2007). Damaged cuticle decreased nematode motility and loss of activity led to nematode death. Cuticle damage occurred together with leakage of the inner materials of the nematode (Luo et al., 2007). The potential of a body or substance to conduct electrical current derives from particles that are positively and negatively charged and its travel inside the substance, resulting in electrical conductance (Jones, 2005). The more free ions present, the higher the conductivity. In our study, both Genistein and Daidzein increased the electrical conductivity of J2s and showed maximum body fluid leaking of *H. glycine* in the early stage of suspension treated. We found that the electrical conductivity is time and dosage-dependent. The outcomes of our study clearly demonstrated that the use of Genistein and Daidzein damaged the cuticle of nematode and caused the leakage of inner materials of the nematodes, compromising the nematode ability to keep the pressure of the whole body, normal body shape, and length.

Due to the restructuring of the body, elongated stylet, and paralysis, respiration reduced, the cuticle was damaged, and then it caused some difficulties for the nematode to travel, locate, and penetrate the host, and remove the nutrient from the soybean root. Consequently, a slower rate of development, abnormal sexual differentiation was established.

In conclusion, both Genistein and Daidzein are safe, effective, and eco-friendly chemicals and isoflavones displayed potential nematicidal against *H. glycines*. The inhibition of the isoflavones to J2 motility, respiration, and body fluid depended on dosage and exposure time. Further studies on mechanisms is required to maximize the control application against SCN.

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## Authors' Contributions

**Conceptualization:** Duan, Y.X.; Zhu, X.F. **Data acquisition:** Ma, Y.Z.; Yuan, R.H. **Data analysis:** Ma, Y.Z.; Yuan, R.H.; Sikandar, A. **Design of methodology:** Ma, Y.Z.; Wang, Y.Y. **Writing and editing:** Sikandar, A.; Wang, Y.Y.

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