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Action of sterol glycosides on *Meloidogyne incognita* infecting tomato and cucumber roots

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Summary. Treatment of tomato and cucumber plants with furostanol glycosides obtained from cell cultures of *Dioscorea deltoidea* Wall. decreased their susceptibility to infection by the root-knot nematode *Meloidogyne incognita*. In treated plants the fecundity of the nematode was decreased five-fold, females were smaller and the sex ratio shifted toward an increase in males.
Key words: tomato, cucumber, induction, furostanol glycosides, *Meloidogyne incognita*, resistance.

Sterol glycosides involved in plant resistance to pathogens are considered as potentially promising alternatives for ecologically safe pest control. Sterol glycosides - derivatives of furostanol and spirostanol - form the group of chemical compounds known as saponins. These compounds are known to possess haemolytic, oncostatic, fungicidal and antibiotic properties (Tschesche & Wulff, 1973). Also, a correlation between the steroid glycoalkaloid "tomatin" and susceptibility of tomato to the root-knot nematode *Meloidogyne incognita* has been demonstrated (Zinovieva, 1989). The reported ability of saponins to repel some arthropod pests (Shany *et al.*, 1970) was explained by their olfactory repellency or by their negative effects on insect-symbiotic fungi (Tschesche & Wulff, 1973). Also, antioxidant (Kintja *et al.*, 1987), immunoregulatory (Madulenko *et al.*, 1989) and other types of biological activity (Paseschnichenko, 1992) were reported for the furostanol glycosides (FG).

Here we present the results of a study on the susceptibility of tomato and cucumber plants treated with furostanol glycosides, obtained from cell cultures of *Dioscorea deltoidea* Wall., to infection by the root-knot nematode *Meloidogyne incognita*.

MATERIALS AND METHODS

The FG preparation used in the experiments (Fig. 1) was extracted from a cell culture of *Dioscorea deltoidea* Wall. (strain IPhR DM-05). This preparation consisted of a 6:4 mixture of protodioscin and deltoside (Vasiljeva *et al.*, 1988). The effect of various methods of FG application on plant and nematode development was assessed with the heterozygous hybrid tomato F_1 Karlson, which is susceptible to *M. incognita* (susceptibility index 30%), and cucumber cv. NIIOKH-42. Plant growth and their infestation with *M. incognita* were recorded as described previously (Zinovieva *et al.*, 1995). All data were analyzed according to standard procedures for analysis of variance. The least significant differences were calculated at $p<0.05$.

Experiment 1. Nematode mobility bioassay. Fifty second stage juveniles of *M. incognita* were treated with 1 ml water FG solution (1-25 mg/ml, 20 °C) and a control batches of nematodes were treated with the same volume of distilled water (Table 1). Three replicates were used for each FG concentration and nematode mobility was assessed at 3, 12, 24, 48, and 72 hours after treatment.

Experiment 2. Tomato seed germination after treatment with different FG concentrations. Twenty tomato seeds were treated in covered glass vials with 2 ml of FG solutions (0.01; 0.1; 0.5; 1.0; 1.5 mg/ml) or distilled water as a control (3 replicates of each treatment). After two hours of treatment the seeds were transferred to 1.5% Difco agar for germination. Root length of the tomato seedlings was measured at 24, 48 and 72 hours post treatment as an indicator of FG activity (Table 2).

Experiment 3. Tomato seed germination and growth after different duration of FG treatments. Ten tomato seeds were treated with 1 mg/ml FG

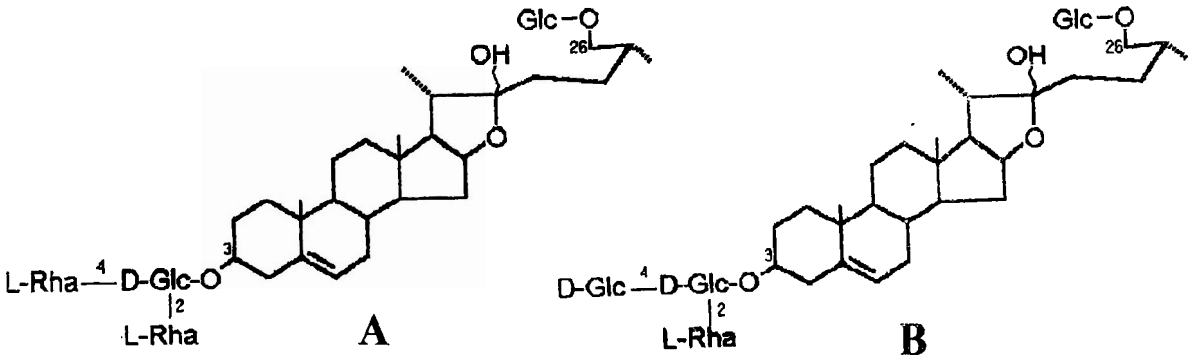

Fig. 1. Chemical structures of protodioscin (A) and deltoside (B).

solution, or distilled water as control, for 0.5; 1; 2; 6; or 24 hours and then planted (3 replicates of each treatment). Treatment longevity as a factor in FG action was assessed by the percentage of germinated seeds and stem length at 21 days after planting (Table 3).

Experiment 4. Effect of preplanting seed treatment with FG on plant and nematode development. Seeds were immersed in FG concentrations of 0.01; 0.05; 0.1; 0.5 and 1.0 mg/l for 2 hours and then planted in 10 cm dia. pots with 500 g of soil in each. Ten plants originating from each FG concentration were infected with 3000 *M. incognita* juveniles (suspended in 1 ml of water) on day 20 after germination (Tables 4 & 5).

Experiment 5. Effect of foliar application of FG on plant and nematode development. Four week-old plants were sprayed with 0.05 and 0.025 mg/ml FG solution (15 ml per plant) and 3000 *M. incognita* J_2 (diluted in 1 ml of water) were added the next day. The foliar application was repeated after 2 weeks. Plants sprayed with the same volume of water and infected with the same numbers of *M. incognita* were used as a control (Table 6). The number and size of galls, female size, number of eggs in the egg sac and plant height were recorded on day 40 after infection. All the experiments were done with 10 replicates.

Experiment 6. FG action on *M. incognita* development in tomato roots. Two week-old tomato plants grown from FG treated seeds (2 hours, 1.0 mg/ml) or from those grown only in distilled water as a control were infected with 3000 *M. incognita* J_2. The penetration and development of *M. incognita* in the roots were observed during the first 7 days after the infection by daily observations of 3 infected plant roots, which were stained with acid fuschin, flattened between two glass cover-slips and examined with a stereoscopic microscope. In the following period the roots were observed every five days. The maturity of *M. incognita* females was identified as egg sack formation. The experiment was terminated 40 days after infection and the adult *M. incognita* were counted. Eggs obtained from the sack envelopes were distributed at 100 per dish and hatching was observed during 5 days at 20 °C to ascertain egg viability using 5 replicates (Table 7).

RESULTS AND DISCUSSION

Experiment 1. The data in Table 1 shows that the visible effect of FG on *M. incognita* mobility was evident only at concentrations > 5.0 mg/ml. All the nematodes were immobilized soon after 72 hours at such FG concentrations.

Experiment 2. Concentrations of FG (0.5 to 1.0 mg/ml) did not significantly increased root length compared with the untreated control (Table 2) but root growth was slightly inhibited at FG 1.5 mg/ml, the highest concentration tested.

Experiment 3. Percent germination of tomato seed was adversely affected when seeds were immersed for 6 hours or more in FG concentrations (Table 3). Two hour immersion of seeds stimulated plant growth but treatment for 6 and 24 hours severely affected plant growth.

Experiment 4. Immersion of the seeds at all FG concentrations reduced the numbers of galls on the roots of the tomato plants (Table 4). However, mean female length was independent of FG concentration and the number of eggs produced per *M. incognita* female was not significantly different between treatments. Similar results were obtained with cucumber (Table 5), but cucumber plant height was minimal after plant treatment with FG concentrations at 0.5 and 1.0 mg/ml.

Experiment 5. Foliar FG application decreased the number of galls on cucumber roots. Gall formation was reduced after the FG foliar application both on tomatoes and cucumbers. A significant reduction of galling was obtained with a single application on tomato plants and with a double application on cucumbers (Table 6).

Experiment 6. Penetration of *M. incognita* juve-

Table 1. FG effect on *in vitro* mobility of *Meloidogyne incognita* juveniles.

FG concentration (mg/ml)	Mobility (number of mobile juveniles as % of their total number)				
	3 hours	12 hours	24 hours	48 hours	72 hours
Control (water)	100	95	90	78	64
1.0	100	97	93	85	69
5.0	100	65	37	24	2
10.0	65	25	5	0	0
25.0	0	0	0	0	0

Table 2. Tomato seed germination after treatment with different FG concentrations.

FG concentration (mg/ml)	Root length (mm) after FG treatment during		
	24 hours	48 hours	72 hours
Control (water)	1.0	2.9	3.8
0.01	1.1	2.9	3.7
0.1	1.0	3.0	3.9
0.5	1.3	3.2	4.3
1.0	2.0	3.5	4.7
1.5	0.9	2.5	3.0
LSD p=0.05	0.3	0.4	0.3

Table 3. Effect of duration of seed immersion in 0.1% FG solution on seed germination and stem length.

Exposition (hours)	Time for germination (days)	Germinated seeds as percent of control	Stem length after 21 days (cm)
0.5	3-4	100	11.2 (11.0)*
1.0	2-5	102	11.6 (10.8)
2.0	2-4	104	21.7** (12.5)
6.0	6-7	83	5.0** (12.4)
24.0	9-11	32	3.9** (12.0)

* - Stem length in control (treatment with water);
** - Significant difference between experiment and control at $p<0.05$.

Table 4. Effect of tomato seed treatments with FG on the *Melodogyne incognita* and host plant height.

FG concentration (mg/ml)	Mean number of galls/ g of roots	*M. incognita* female size (mm)	Mean number of eggs/ female	Plant height (cm)
0.01	250	0.270	146	72.5
0.05	166	0.313	117	70.3
0.1	180	0.265	123	71.3
0.5	180	0.284	135	73.1
1.0	84	0.221	108	114.6
Control (water)	296	0.309	166	73.6
LSD	41.7	0.039	39.3	11.7

niles into the plants was retarded after FG application (Table 7). The first juveniles were found in roots 1 to 3 days after the time of their appearance in the control plants. Mature females were found in treated plants 5 to 7 days after those in the controls. Also, there were fewer females with egg sacks in FG treated plants and the viability of eggs was decreased. An unexpected result was the shift of sex ratio in *M. incognita* populations in treated plants.

The treatment of tomato or cucumber seeds, or foliar applications, with oligoglycosides at appropriate concentrations, reduced the invasion of roots by *M. incognita* and suppressed the development of the nematode. The effect on *M. incognita* was achieved at concentrations of FG that had no effect on the nematode *in vitro,* suggesting that the action of FG is mediated by the plant and enhances the natural defense mechanism. Thus, FG offers the prospect of an environmentally friendly means of nematode control.

Table 5. Effect of cucumber seed treatments with FG on the *M. incognita* and host plant height.

FG concentration (mg/ml)	Mean number of galls/ g of roots	*M. incognita* female size (mm)	Mean number of eggs/ female	Plant height (cm)
0.01	300	0.330	106	78.3
0.05	388	0.270	121	70.8
0.1	322	0.280	168	68.2
0.5	344	0.290	156	55.6
1.0	322	0.290	169	53.4
Control	564	0.330	174	72.3
LSD	41.7	0.054	42.4	14.1

Table 6. Effect of FG foliar application on root galling and number of eggs of *Meloidogyne incognita*.

Plant	FG conc. mg/ml or water as control	Number of treatments	Gall reduction as percent of control	Mean egg number/female
Tomato	Control (water)	–	–	254.6
Tomato	FG 0.05	1	60	146.1
				LSD ($p<0.05$) = 37.4
Cucumber	Control (water)	–	–	434.8
Cucumber	FG 0.05	1	24	347.3
Cucumber	FG 0.025	2	65	299.5
				LSD ($p<0.05$) = 81.3

Table 7. Effect of FG on *Meloidogyne incognita* development in tomato roots.

FG concentration (mg/ml)	Penetration time (days)	Maturation time (days)	Sex ratio male/ female	Number of adult females (%)	Egg hatching (%)
Control	4-6	16-21	2:100	84	72
1	5-7	23-27	10:100	76	58

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Зиновьева С. В., Удалова Ж. В., Васильева И. С., Пасешниченко В. А. Воздействие стероидных гликозидов на нематод *Meloidogyne incognita*, поражающих корни томатов и огурцов.

Резюме. Обработка томатов и огурцов гликозидами фуростанолового ряда, полученными из культуры клеток *Dioscorea deltoidea* Wall., снижала их восприимчивость к заражению галловыми нематодами *Meloidogyne incognita*. В обработанных растениях плодовитость нематод снижалась в 5 раз, самки были мельче, соотношение полов смещалось в сторону преобладания самцов.