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THE EFFECT OF RADIATION ON DEVELOPMENT OF ROOT-KNOT NEMATODES

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Abstract

Root-knot nematodes (*Meloidogyne* spp.) are one of the most important plant parasitic nematodes of great economic importance which reduce the quantity and the quality of the yields of many cultivated and wild plants everywhere (in tropical, subtropical and temperate regions). The objectives of the study were to investigate the changes in the *Meloidogyne arenaria* and its host Tiny Tim tomato plant under radiation influence The influence of various doses of γ-irradiation (90, 700 and 1800 mGy) on cv. Tiny Tim tomato plants and developing eggs sacs of root-knot nematode *Meloidogyne arenaria* were investigated. Ionizing radiations of tomato seeds by low dose (90 mGy) stimulate development of plants. High doses of γ-irradiation (700 and 1800 mGy) suppress development (height, root and shoot weight) of tomato plants. High irradiation doses (700 and 1800 mGy) retarded the growth of nematodes. Metric characteristics of *M. arenaria* females, mainly body size, were smaller. The highest experimental dose (1800 mGy) prevented the development of females of *M. arenaria* (J4) to mature forms. A change of female to male ratio under the influence of γ-ionizing radiation has been observed, resulting in a decrease in males. These results show aspects for future research into the application of γ-irradiation in management of root-knot nematodes.

***Keywords*:** γ-irradiation nematodes; *Meloidogyne arenaria;* cv.Tiny Tim; tomato plants.

Introduction

Parasitism as an interrelation between two subjects and is distributed among all alive organisms-plants, invertebrate and vertebrate animals. Root-knot nematodes (*Meloidogyne* spp.) are one of the most important plant parasitic nematodes of great economic importance. As a root-knot nematodes, the obligate endoparasite *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 are economically very important plants parasites which reduce the quantity and the quality of the yields by causing formation of galls on the host plant roots and consequently disruption of their vascular system. They are pests for many cultivated and wild plants everywhere (in tropical, subtropical and temperate regions). Losses due to these parasites are estimated at hundreds of dollars annually and their adaptation to parasitism is so perfect that in many cases control strategies nave been unsuccessful [17].

At present the number of species belonging to *Meloidogyne* is about 80. They infect more than 2000 plant species [12]. *Meloidogyne* invasion causes heavy qualitative and

quantitative damages of the plant production up to 70-80% in many countries including Bulgaria [16]. The numerous species belonging to *Meloidogyne*, their ecological and biological abilities to survive under unfavorable conditions make their control very difficult. The contemporary requirements to the quality and quantity of the plant production as well as to the preservation of the environment strongly restrict use of pesticides. Elaboration development and probation of new methods for control (suppression of the nematode development and reproduction) are necessary.

The parasitology could be an area where the ionizing radiation could have an application and could be used for control of the parasites [6]. Nematode species vary in their sensitivity to irradiation [8, 11, 13]. It is proved that the irradiation retarded the growth of nematodes according to the dose used. Irradiation of second stage juveniles (J2) retarded the growth more that the irradiation of later-stage juveniles. In 1986 the Chernobyl accident gave a wide field for studies in the Chernobyl accident zone of the parasites and parasitic systems in radiation biocenosis. Since the accident the regular studies on the parasitic system have been carried out in the Chernobil accident zone [2].

It is known that γ-irradiation in small doses stimulate increases the germination rate and seed vigour, growth and development, the processes of respiration and photosynthesis of plants, and, ultimately, to improve their productivity [7]. This is due to changes in the metabolism of germinating seeds exposed at the earliest stages of development — accelerated mobilization of nutrients, increased oxidative processes, changes to the nucleotide composition of DNA and RNA [5]. Under the influence of high dose γ-oppressive industrial irradiation slowdown comes the appearance of ugly forms and subsequent destruction of plants. This is due to the formation of free radicals, reduce the content of nucleic acid, DNA mutations, oxidative stress is a significant activation of oxidative processes affecting and enzyme systems. Inhibition of growth also was associated with inhibition of the synthesis of auxin-physiological hormones growth, but have not been finally clarified the mechanisms of action of radiation on synthetic auxins IAA, inter alia, promoting cell growth through stretching [1].

Our previous investigations established the differences in using α- and γ- irradiation on some parasitic nematodes [19, 10].

The present work has a purpose to investigate the influence of increasing doses of γ-radiation on the life cycle of root-knot nematode *M. arenaria* and on its host – tomato plants, cv. Tiny Tim.

Material and methods

The experiments were carried out under laboratory conditions. Population of plant-parasitic nematode *M. arenaria* was used in the experiment. Egg sacks with eggs containing larvae (J2) were put into small test-tubes. Every test-tube contained 10 egg sacks. The tubes were irradiated.

For study of irradiation effect on Tiny Tim tomato plants, seeds were irradiated with the same doses of gamma rays. The seeds were sown in soil sterilized by heating under laboratory conditions (t = 20° C). The planting of the irradiated seeds was made on the next day after irradiation. The development of the tomato plants was observed during the following 60 days. The experiment was carried out in three replications.

The irradiation was performed by using a source of gamma-rays was ^{60}Co with activity of 281 μCi and dose rate of 0.1mGy/h. The dosage of γ-irradiation used in this experiment included 90, 700 and 1800 mGy. The parasites and seeds were given a single γ-irradiation treatment.

Results and discussion

Influence of γ-irradiation on development of the nematodes M. arenaria.

Root-knot nematodes spend most of their active lives within plant roots, feeding on dramatically modified host cells. Their life cycle involves passage through a series of four juvenile stages, separated by molts, during which the cuticle is replaced. The infective stage is the motile, juvenile (J2) that penetrates the root and migrates to a site near the vascular tissue to establish a permanent feeding site. After feeding is initiated, the nematode becomes sedentary and then undergoes three molts during development to the adult stage. Adult females are bulbous and nonmotile. Egg production begins at 3 to 6 weeks after the initial infection, depending on the species and environmental conditions. Gender is determined epigenetically, with males increasing in frequency under conditions of crowding or poor nutrition [18]. Males also pass through a nonmotile developmental stage but regain motility during the third molt before leaving the root. After development of the female eggs are released on the root surface in a protective, gelatinous matrix (egg sack).

In Figure 1 the data on hatching larvae from eggs in the control and experimental variants (the number of investigated eggs for every variant is 400).

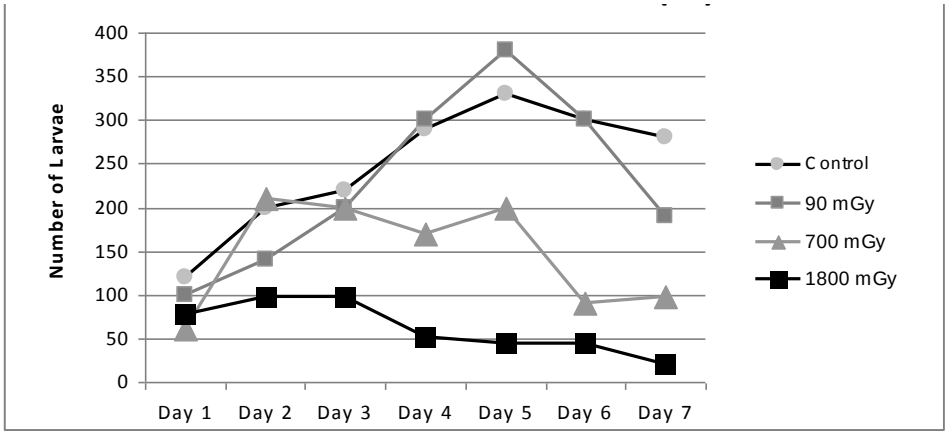

Figure 1. Effect of different doses of irradiation on hatching larvae

The data obtained have shown that irradiation effect on of the larvae hatching. The number of the larvae hatching in control variant (nonirradiated) sharply increased the first day, reached maximum on the fifth day and slowly decreased to the seventh day. Hatching of the eggs irradiated with 90 mGy in general follows the trends of the control variant (Fig 1). Irradiation with 700 mGy leaded to considerable breaking of the egg hatching rhythm compared with the control. Irradiation with 1800 mGy strongly decreased egg hatching to the end on the seventh day.

Metric characteristics of males and females of *M. arenaria* are made after De Man indices. For statistical reliability each variant was performed in three repetitions. Effect of gamma rays on the metric characteristics of nematodes is given in Table 1, 2.

Table 1.

Metric characteristics of *M. arenaria* (males)

Measurments	Experimental variants

	Control (N=20)	90 mGy (N=20)	700 mGy (N=15)	1800 mGy (N=0*)
L (body length)	1,33 (1,20–1,90)	1,40 (1,25–1,90)	1,12 (0,95–1,90)	*
a (body length/ body diameter)	57 (42–61)	55 (43–65)	50 (40–67)	*
b (body length/ oesophagus)	12,5 (11,8–15,4)	13,0 (12,1–16)	12 (11,0–17)	*
c (body length/cauda)	8,5 (7,1–10,3)	9 (7,5–11,2)	10 (7,0–13)	*
st (length of stylet)	21 (19,5–22,1)	21 (20–23)	19,5 (18–22)	*

** males are not found*

Treatment with 90 mGy does not affect on metric characteristics of males. Irradiation with 700 mGy evidently reflexes on metric characteristics of males (shorter body length and larger variations of the metric values). Irradiation with 1800 mGy stops the development of male specimens (Tab. 1).

Higher doses of gamma rays (700 and 1800 mGy) influence on metric characterisrics of *M. arenaria* females, mainly on body size (after irradiation with 700 mGy body diameter occurs lower values and female body is more elongate).

The highest experimental dose (1800 mGy) destroys development of female specimens (J4) to mature forms (Tab. 2).

Table 2

Metric characteristics of *Meloidogyne arenaria* (females)

Measurments	Experimental variants			
	Control N=20	90 mGy (N=20)	700 mGy (N=20)	1800 mGy (N=20)
L (body length)	0,780 (0,630–0,850)	0,800 (0,700–0,900)	0,700 (0,580–0,800)	*
d (body diameter)	0,430 (0,400–0,520)	0,400 (0,350–0,480)	0,350 (0,300–0,500)	*
st (length of stylet)	21 (19,5–22,1)	21 (20–23)	19,5 (18–22)	*

**Only J4 were found*

2. Data for the characterization of the Tiny Tim plants

During the experiment a data were recorded on the following trials on the parameters of plants – height (cm), shoot weight and dry weight of the experimental plants, root and shoot weight and dry weight. The data obtained for the height changes of Tiny Tim control and experimental plants are shown in Table 3.

Table 3

Root and shoot weight and dry weight of the plants

Irradiation dose, mGy	Root weight, g	Shoot weight, g	Dry weight, g	
			roots	shoots
Control plants	25,3±1,9	46,7±3,5	17,5±1,4	22,3±1,9
90 mGy	27±2,5	43±3,9	18±1,3	22±2,1
700 mGy	17,5±1,5	22,8±2,0	9,0±0,7	12±1,1
1800 mGy	11,5±1,0	16,0±1,2	7,0±0,5	10,5±0,9

Dose of 90 mGy does not affect growth of the plants. Considerable differences were found in variant irradiated with dose of 700 mGy and the most negative effect after treating the seeds with gamma rays were observed in variant with dose of 1800 mGy.

The sensitivity of plants to ionizing radiation depends of radiation doses. Very few doses have been shown to stimulate plant growth [14]. High doses of radiation disturb the synthesis of DNA, RNA and protein and also enzyme activity [15]. It is proved that seeds irradiated with lower doses (0,5 kGy) showed 10% germination. Radiosensitivity varies from species to species even among genotypes of the same species [4].

Another parameters which have been measured are root and shoot weight (Tab. 3). Depending on the radiation dose the root and shoot weight has changed. With the increasing of doses the weight of the root and shoot decreases.

Change of correlation between females and males specimens under influence of ionizing radiation to decreasing of males is proved in the experiment. Decreasing of male numbers is strongly depended on irradiation doses [2]. It is also proved that males of helminths are more sensitive to ionizing radiation than females. Increasing of ionizing radiation doses leads to considerable decreasing of males [3]. Irradiation of eggs (Ascarids) with 60–80 kR decreases their embrional development and 200 kR absolutely stops migratory activity of the larvae [2]. Irradiation of the seeds of mung been with gamma rays increments plant height and weight and suppress infection of the roots with plant pathogenic fungi [15].

Conclusion

The great number of root-knot nematodes (*Meloidogyne* spp.), their worldwide distribution and economic importance require their detail investigation in different aspects.

The irradiation retarded the growth of nematodes in proportion to the doses as higher doses of gamma rays (700 and 1800 mGy) influence on metric characterisrics of *M. arenaria* females, mainly on body size (after irradiation with 700 mGy body diameter occurs lower values and female body is more elongate. Change of correlation between females and males specimens under influence of γ -ionizing irradiation has been observed and is express in decreasing of males. The highest experimental dose (1800 mGy) destroys development of female specimens of *M. arenaria* (J4) to mature forms. The number of incubated J2 forms controls and irradiated with 90 mGy sharply increased after the first day after irradiation, reached maximum on the fifth day and slowly decreased to the seventh day. Irradiation with 700 mGy leaded to considerable breaking of the egg incubation rhythm compared with the controls. Irradiation with 1800 mGy strongly decreased incubation to the end on the seventh day.

Experimental investigations of ionizing radiation effect on the host plants show the seeds effect of high doses gamma rays. Ionizing radiation at low doses stimulate the development of plants. The present investigation established that γ-irradiation in doses (700 and 1800 mGy) suppress the development (height, root and shoot weight) of host plants.

Investigations of the effect of ionizing radiation on the host-parasite systems make clear intime interactions between parasite and its host and is of great importance to increase the plant resistance to parasitic attacks. Detail studies of these processes are important to understand number of changes in the nature and on this base to work out programs for prevention of the environment.

Irradiation may be an alternative way to fumigation for desinfectation of plant material. These results show that up-regulation of some physiological characteristics and seedling growth of wheat which follow gamma radiation treatment may be used for abiotic control such as drought and salt stress.

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ДЕЙСТВИЕ РАДИАЦИИ НА РОСТ И РАЗВИТИЕ КОРНЕВЫХ НЕМАТОД

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Реферат

Корневые нематоды (Meloidogyne spp.) являются основными представителями класса нематод, паразитирующих на растениях. Нематоды наносят большой экономический ущерб, который выражается в снижении продуктивности и ухудшении качества урожаев сельскохозяйственных культур и дикорастущих растений, произрастающих во всех климатических зонах (тропических, субтропических, а также в регионах с умеренным климатом).

Цель настоящего исследования состоит в изучении изменений, происходящих в галловой нематоде *Meloidogyne arenaria* и ее хозяине – растении томатов Tiny Tim («Крошка Тим») под воздействием радиации.

Было изучено влияние различных доз гамма-излучения – 90, 700 и 1800 мГр на коэффициент изменчивости растений томатов Tiny Tim и развитие яиц галловой нематоды *M. arenaria*. Ионизирующее излучение в малых дозах (90 мГр) стимулирует рост и развитие растений, в то время как высокие дозы гамма-излучения (700 и 1800 мГр) подавляют развитие томатов, замедляя скорость роста побегов и корней.

Использование ионизирующей радиации в высоких дозах (700 и 1800 мГр) способствует замедлению роста нематод. По метрическим данным, в основном это сказывается на уменьшении размеров тела самки *M. arenaria*. Применение самой высокой экспериментальной дозы излучения (1800 мГр) препятствовало переходу самок *M. arenaria* (J4) ко взрослой стадии.

Наблюдались изменения численного соотношения самцов и самок под влиянием гамма-излучения, приводящие к снижению числа самцов.

Данные результаты открывают перспективы для дальнейших исследований воздействия гамма-излучения на развитие корневой нематоды.

Ключевые слова: гамма-излучение; нематоды; *Meloidogyne arenaria*; коэффициент изменчивости; Tiny Tim; растения томатов.