Root-Knot Nematodes (*Meloidogyne* spp.) Infestation and Reproduction on Soybean (*Glycine max* L. Merrill) Treated with Varying Doses of Gamma Rays

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Received: 10th October 2019, Accepted: 28th April 2020

Abstract
A study was carried out on a root-knot nematode (RKN), naturally infested field at the Department of Agronomy, University for Development Studies, Nyankpala campus during the 2017 major cropping season. The objective was to evaluate the potential of root-knot nematodes to infest and reproduce in soybean cultivar 'Jenguma' treated with different doses of gamma rays. The experiment consisted of 5 treatments viz- soybean seeds irradiated with 150 Gy, 200 Gy, 250 Gy, 300 Gy and 0 Gy doses of gamma rays. The treatments were replicated three times in a randomized complete block design. Yield of soybean and reproduction index of RKN were determined at 10 WAP. Results of the study showed that soybean seeds irradiated with 150 Gy recorded maximum fresh and dry root weights of 24.32 g and 0.90 g respectively whilst treatment 300 Gy recorded the lowest mean fresh and dry root weights of 15.00 g and 0.80 g respectively. Plants treated with 150 Gy produced crops with heavier grains of 4.65 t/ha compared to 1.37 t/ha produced by unirradiated seeds. Seeds treated with 150 Gy had the least root galls, fewer number of second stage juveniles of root-knot nematodes and lowest reproduction index of 0.47, 44 and 8.46, respectively, compared to other treatments. The study therefore concluded that treating soybean var 'Jenjuma' with a dose of 150 Gy increased its resistance to RKN infestation and hence suggested for use in the management of root-knot nematodes in soybean production.

Key words: Gamma irradiation, root-knot nematodes, reproductive index, resistance, soybean

Infestation et Reproduction des Nématodes Meloidogyne (*Meloidogyne* spp.) Sur le Soja (*Glycine max* L. Merrill) Traité à Des Doses Variables de Rayons Gamma

Résumé
Une étude sur le terrain a été réalisée sur un nématode meloidogyne (RKN en anglais), champ naturellement infesté au Département d'agronomie de l'Université des études Du Développement du campus de Nyankpala pendant la grande saison agricole 2017. L'objectif était d'évaluer le potentiel d'infestation et de reproduction des nématodes meloidogyne dans le cultivar de soja « Jenguma » traité à différentes doses de rayons gamma. L'expérience a consisté en 5 traitements

à savoir: graines de soja irradiées avec 150 Gy, 200 Gy, 250 Gy, 300 Gy et 0 Gy de rayons gamma. Les traitements ont été reproduits trois fois dans un modèle de bloc complet randomisé. Le rendement du soja et l'indice de reproduction du RKN ont été déterminés à 10 WAP. Les résultats de l'étude ont montré que les graines de soja irradiées avec 150 Gy ont enregistré des masses maximales de racines fraîches et sèches de 24,32 g et de 0,90 g respectivement, tandis que le traitement 300 Gy a enregistré les masses moyennes de racines fraîches et sèches les plus faibles de 15,00 g et de 0,80 g respectivement. Plantes traitées avec 150 Gy produit des cultures avec des grains plus lourds de 4,65 tonnes par hectar contre 1,37 tonnes hectare produites par des semences non irradiées. Les graines traitées avec 150 Gy présentaient le moins de galles radiculaires, moins de juvéniles au deuxième stade de nématodes meloidogyne et le plus faible indice de reproduction de 0,47, 44 et 8,46, respectivement, comparativement à d'autres traitements. L'étude a donc conclu que le traitement de la variété de soja « Jenjuma » avec une dose de 150 Gy augmentait sa résistance à l'infestation de RKN et suggérait donc son utilisation dans la lutte contre les nématodes meloidogyne dans la production de soja.

Mots-clés: *Irradiation gamma, nématodes meloidogyne, indice de reproduction, résistance, soja*

Introduction

Soybean (*Glycine max* L. Merrill) is an important leguminous crop that grows globally in the tropical, sub-tropical and temperate climates (Liebenberg, 2012). The crop is gaining prominence in Northern Ghana largely due to its multi-purpose usage for oil extraction (Rheenen *et al.,* 2012). It contains proteins, provides high levels of essential amino acids except methionine and cystine, rich in poly unsaturated fatty acids especially omega-6 and omega-3 fatty acids, high total minerals and carbohydrates (Hafiz and Tyagi, 2013).

The production of soybean is, however, threatened by the activities of plant-parasitic nematodes (Torr *et al*., 2007). Of all the plant parasitic nematodes associated with soybean, root-knot nematode (RKN), *Meloidogyne incognita* is the most devastating nematode pest accounting for about 30-50% yield loss (Khalil, 2013) and over US$500 million in revenue loss in soybean production Keren-Zur *et al*. (2000).

Soybean plants infested with root-knot nematodes show typical symptoms of root galling, leaf chlorosis and frequent wilting in plants. The number and sizes of galls induced by RKN varies depending on the susceptibility of the cultivar, population density of the nematode and favorable environmental conditions (Dias *et al*., 2009). RKN-infested roots change their nutrient and water uptake, leading to decreased yield.

Protecting plants against nematodes is difficult because nematodes cannot be completely eradicated from the soil (Budai *et al.,* 2005). It is therefore, important to control the potential rise of root-knot nematodes population before it reaches economic threshold (Viaene *et al.,* 2013). Although nematicides are highly effective in nematodes management, indiscriminate use of chemicals results in phytotoxicity in soybean crops, environmental pollution and nematodes resistance (Sikora *et al*., 2005). Nematicides are also highly toxic to man and animals when not properly used (Abawi and Widmer, 2000). Exploitation of resistance in crops is one of the most environmentally safe, effective and cheapest components of integrated pest management (Rosa *et al*., 2013). Resistance traits increase crop yield in the presence of nematodes (Rosa *et al*., 2013). In a resistant variety, nematodes fail to develop and reproduce normally within root tissues, allowing plants to grow and produce fruits even though nematode infection of roots occurs (Friedman and Baker, 2007).

High levels of intraspecific variations within *Meloidogyne* genome, play important role in their capacities to reproduce in certain hosts (Moens *et al*., 2009).

Mutation have successfully been used in breeding to develop and generate genetic variations in crops varieties through chemical and physical mutagenesis (Lundqvist *et al*., 2012; Shanthala *et al*., 2013) to obtain desired traits. It has been used as an effective and non-chemical means to control pest and disease in crop production (Hallman, 2011). Observed differences in agronomic traits such as earliness, number of productive tillers, seed weight and yield components in millet and soybean treated with different doses of gamma radiations (Addai and Salifu, 2018; Addai, 2019) show the potential of using radiations to create genetic variations in crops to obtain desired results. Hermelin *et al*. (1987) reported a reduction in germination time in sunflower mutant lines and attributed this to the effects of irradiation. Application of gamma radiations have successfully been used to manage biotic stress in crops. As a pest and disease control tool, Chu *et al*. (2015) reported that application of gamma radiations at a dose of 4.0 kGy completely inhibited growth of *Botrytis cinerea* on rose flowers. In another studies, Bhagwal and Duncan (1998) reported that mutant lines of banana showed tolerance to *Fusarium oxysporum* f. sp. *cubense* whilst Myers *et al*. (2018) reported that second stage juveniles of *Rotylenchulus reniformis* irradiated with 100 Gy and 300 Gy could not successfully reproduce after inoculation on *Ipomea batatas*. Similarly, Chinnasri *et al*. (1997) observed that application of gamma radiation dose of 7.5 kGy killed all second-stage juveniles (J2) of *Meloidogyne javanica* within 24 hours after treatment and that egg hatch was completely inhibited at 6.25 kGy The option of treating soybean with gamma rays to improve its resistance as a management strategy against RKN infestation has not fully been explored in Ghana. The objective of the study was to evaluate the potential of root knot-nematodes to infest and reproduce in soybean cultivar 'Jenjuma' treated with different doses of gamma rays.

Materials and Methods

Study Site

The study was conducted at the experimental field of the Faculty of Agriculture, University for Development Studies (UDS) during the 2017 cropping season. The field is located in the Guinea Savannah Agro-ecological Zone. The experimental site is located on longitude 0^o 58' W and on latitude 9^o 25' N, with an altitude of 183 m above sea level. The area experience a unimodal rainfall pattern ranging from 1000 mm to 12000 mm. The average minimum and maximum temperatures are 25˚C and 35˚C respectively (Lawson *et al*., 2013). Soils of the area are moderately drained and are free from concretions. The soils are shallow with hardpan under the top few centimeters and were derived from Voltaian sandstone. The soils, according to FAO (1988), are classified as Nyankpala series or Plinthic Acrisol.

Source of Soybean Seed and Seed Irradiation / Treatment

Gamma irradiated seeds were obtained from the Department of Agronomy, University for Development Studies, Nyankpala Campus. Seeds of soybean variety Jenguma were exposed to gamma ray doses of 150, 200, 250 or 300 Gy from 60 Cobalt source at the Biotechnology and Nuclear Agricultural Research Institute of the Ghana Atomic

Energy Commission, Accra, Ghana.

Initial Soil Nematodes Population and Experimental Design

Pre-planting soil sampling and nematodes extraction using the modified Baermann tray method showed an average of 520/200 cc second stage juveniles (J2) of *Meloidogyne* species. Based on this, initial RKN population, the field was considered RKN infested. The experiment was mounted on randomized complete block design (RCBD) with five treatments replicated three times. The treatments were; 0 Gy (unirradiated control), 150 Gy, 200 Gy, 250 Gy and 300 Gy. The field was initially planted with tomato var. Pectomech which is reported to be susceptible to root-knot nematodes (Kankam and Adomako, 2014). There was a total of 15 plots, 5 plots per block and each plot measured 3 m x 3 m (9 m^2).

Data Collection

Plant growth data were collected at four, six and eight weeks after planting (WAP) whilst yield data was collected at 10 WAP. At each period, 10 plants were randomly selected from each plot. Agronomic parameters such as number of leaves, number of branches, number of days to 50% flowering, root weight, 100 seed weight and total grain yield were collected. At 10 WAP, soybean plants were uprooted and roots assessed for the presence of root galls. Soil and root samples were also collected and RKN extracted to determine root-knot nematodes second stage juveniles final population.

Assessment of Root-Knot Nematode Galls on Soybean Roots

Soybean roots were washed separately and dried with tissue paper. Root galling was determined using a rating chart by Bridge and Page (1980) on a scale of 0 – 10: 0 = No knots on roots; 1 = Few small knots difficult to find; 2 = Small knots but clearly visible. Main roots clear; 3 = Some large knots visible. Main roots clear; 4 = Larger knots predominate, but main roots clear; 5 = 50% of roots infested; Knotting on parts of main roots; Reduced root system; 6 = Knotting on main roots; 7 = Majority of main roots knotted; 8 = All main roots knotted. Few clean roots visible; 9 = All roots severely knotted. Plant usually dying; 10 = All roots severely knotted; no root.

Soil Sampling and Root-Knot Nematode Extraction

Ten soil cores were sampled before planting and at 10 WAT from depths of 15 cm and 30 cm and bulked together using a 5 cm diameter soil auger from 10 randomly selected sites. Extraction of second stage juveniles (J2) was done using the modified Baermann tray method (Whitehead and Hemming, 1965). Each soil sample was mixed thoroughly and 200 cm^3 of each soil samples was placed into a plastic sieve plate, lined with 2-ply tissue paper. Enough water to soak the soil in the plastic plate was gently added. The set-up was left undisturbed for 48 hours before decanting the nematodes-water suspension into a beaker and water added to obtain a total volume of 250 ml. An aliquot (1 ml) of the nematodes-water suspension was drawn with a 2.5 ml syringe after it has been homogenized for nematode counts under a light microscope. The counting process was repeated three times and the average (520 J2/plot) determined for the initial population. The total number of J2 in 1 ml aliquot was multiplied by the total volume of nematodes-water suspension to obtain the total number of J2 in the suspension.

Data Analysis

Root-knot nematode count data was square root transformed ($\sqrt{\ }$ +0.5) to improve homogeneity of variance. All data collected were subjected to analysis of variance (ANOVA). Genstat (18th edition) statistical package was used in the analysis. Means were separated using the Least Significant Difference test (LSD) at 5% probability level. Reaction of treatments to J2 attack was based on reproduction index (RI) which was calculated as the final J2 extracted from the soil divided by the initial J2 from the soil multiplied by 100 (Taylor, 1967). Reproduction index rating was as follows: RI = 0 (Immune), RI < 1 (Highly resistant), 1 ≤ RI < 10 (Very resistant), 10 ≤ RI < 25 (Moderately resistant), 25 ≤ RI < 50 (Slightly resistant), RI > 50 (Susceptible) (Taylor and Sasser, 1978).

Results

Number of Leaves

There was a significant difference ($P < 0.05$) in the number of soybean leaves at 8 WAP. Soybean seeds treated with 200 Gy recorded the highest number of leaves compared to all the other treatments. The number of leaves in the control (0 Gy) was significantly different from 150 Gy and 200 Gy but not significantly different ($P > 0.05$)) from that of 250 Gy and 300 Gy (Fig. 1).

Number of Branches

There were significant differences ($P < 0.05$) in the number of branches among the various treatments at 6 WAP. However, at 8 WAP, no significant difference in the number of branches was observed between the control (0 Gy) and 300 Gy. Treatments 150 and 200 Gy were significantly different from those of the unirradiated control, 250 and 300 Gy (Fig. 2).

Days to 50% Flowering

Plants reached flowering at different days after planting. There was however, no significant difference ($P > 0.05$) in the number of days to 50% flowering. Treatment 0 Gy used longer days (57.67 days) to reach maximum flowering phase compared to treatments 300 Gy, 150 Gy , 250 Gy, and 200 Gy which used (56.67, 54.67, 54.33 and 53.33 days), respectively (Fig. 3).

Root Weight, 100 Seed Weight and Total Grain Yield

There were significant differences among the treatments in both dry and fresh root weights.

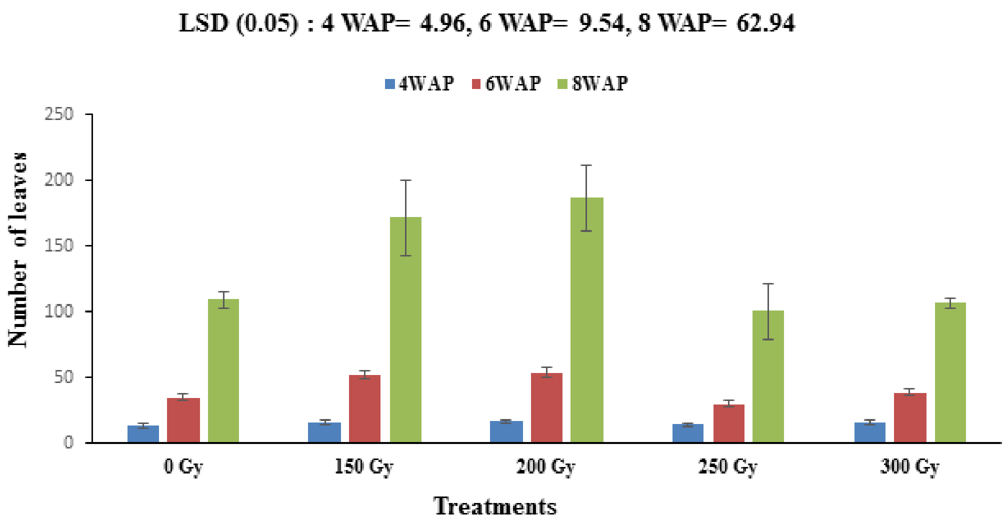

Figure 1: Number of leaves of soybean seeds treated with different doses of gamma radiations

Treatment 150 Gy recorded the maximum mean fresh and dry root weights of 24.32 g and 0.90 g whilst treatment 300 Gy recorded the lowest mean fresh and dry root weights of 15 g and 0.80 g, respectively (Table 1). The 100 seed weight of soybean was significantly different ($P < 0.05$) among treatments (Table 1). Plants treated with 250 Gy recorded the highest seed weight of 12.17 g which was significantly different ($P < 0.05$) from 9.90 g recorded for seeds treated with 0 Gy radiation (Table 1). Treatment 150 Gy recorded the highest grain yield of 4.65, whilst 0 Gy recorded the lowest grain yield of 1.37 t/ha (Table 1).

Root Galling and Root-Knot Nematode Population

There were significant differences in mean root galling at 10 WAP among treatments. Treatment 150 Gy, recorded the lowest mean gall score of 0.47 compared to 5.50 recorded for treatment 300 Gy (Table 2). Similarly, treatment 150 Gy recorded the lowest number of second stage juveniles (J2) per 200 cm^3 of soil of 44 whilst treatment 300 Gy recorded the highest J2 population of 786 (Table 2). The RI recorded for under different treatments varied significantly ($P < 0.05$). The highest RI of 151.15 was recorded for 300 Gy whereas 150 Gy had the lowest RI of 8.46 (Table 2). Treatment 150 Gy showed higher resistance whereas 200 Gy showed moderately resistance to RKN infestation and reproduction (Table 2). However, treating soybean with 250 Gy, 300 Gy and 0 Gy (unirradiated control) radiation dosages were not able to provide resistance to RKN infestation.

Discussions

The present study shows that, treating soybean var 'Jenguma' seeds with different doses of gamma radiations can reduce RKN infestation and reproduction and that at a dose of 150 Gy, the resistance potential of Jenguma increased contradicting Myers *et al*. (2018) who recommended a dose of 300 Gy to inhibit activities of *R. reniformis* on *Ipomea batatas*. RKN resistant plants inhibits the pest's ability

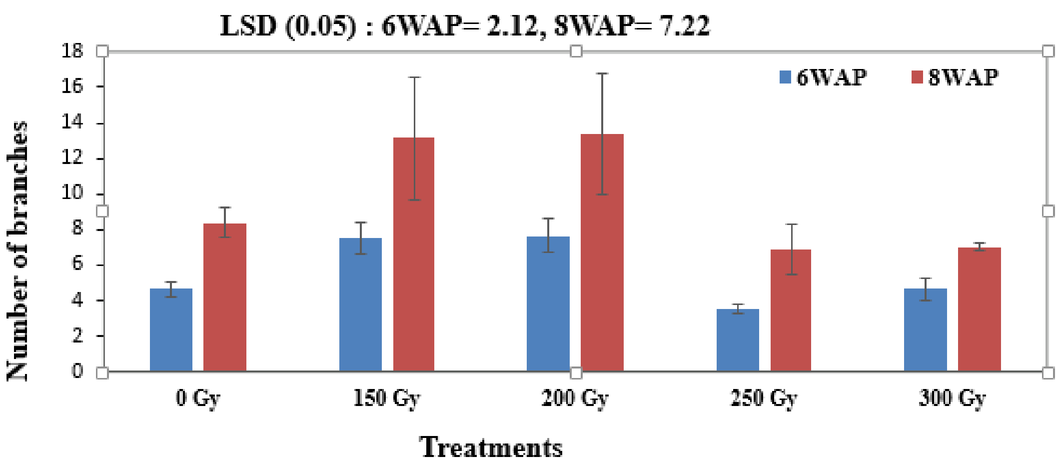

Figure 2: Number of branches of soybean seeds treated with different doses of gamma radiations

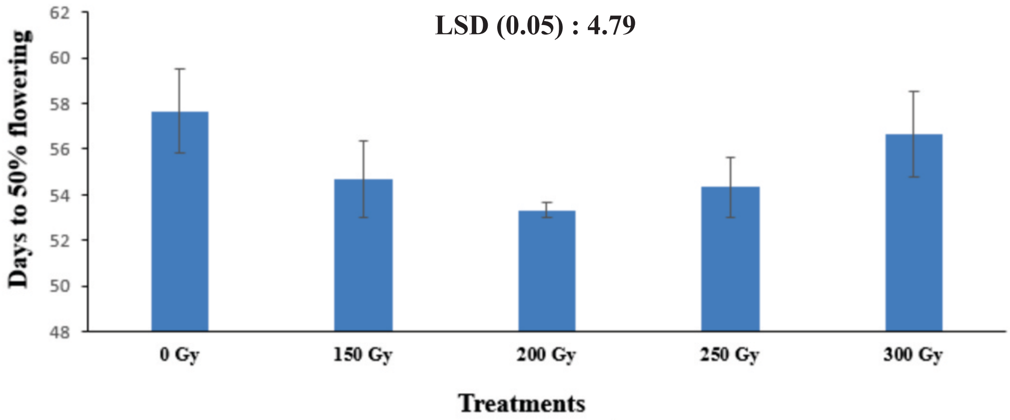

Figure 3: Days to 50% flowering of soybean seeds treated with different doses of gamma radiations

Table 1: Mean fresh and dry root weight, 100 seed weight and total grain yield of soybean plant treated with different doses of radiations at 10 WAT

Treat-ments	Fresh root weight (g)	Dry root weight (g)	100 seed weight (g)	Total grain yield (t/ha)
0 Gy	16.30c	0.90c	9.99c	1.37c
150 Gy	24.32a	1.40a	12.05a	4.65a
200 Gy	21.30b	1.10b	11.48b	4.13b
250 Gy	23.30a	1.30a	12.17a	4.61a
300 Gy	15.00d	0.80c	0.93b	3.88b
LSD (0.05)	1.15	0.12	10.66	0.28
CV (%)	3.20	6.40	3.80	4.70

Column means followed by the same letter(s) are not significantly different at $P > 0.05$ in a least significant difference test.

to multiply to levels it can cause damage (Roberts, 2002) although the plants roots may be invaded. Plants ability to resist stress can be enhanced through radiations as according to Kunz *et al*. (2006), radiations have the potential of increasing plants cellular immunity to pathogens. He *et al*. (2018) reported that treatment of rice leaves with UV-B radiation increased its resistance mechanism and inhibited growth of *M. oryzae*. At higher doses, however, these radiations may be lethal (Mudibu 2012) and can reduce genome stability, growth and productivity of the plant (Kunz *et al*., 2006) due to damages caused to the nucleic acids, proteins and membrane lipids. This might explain why at higher doses, the infestation and reproduction of RKN was high. RKN suppressive potential of the mutant soybean used in the present study compared to the control contradicts Teixeira *et al*. (2016) and Mendy *et al*. (2017) who reported that mutant lines of Arabidopsis could not suppress infestation and reproduction of root-knot nematodes.

Treating seeds with different doses of radiations also positively influenced measured agronomic traits such as number of leaves, fresh and dry shoot weight, flowering and maturity in soybean var Jenjuma compared to the control plants. These findings agree with Addai (2019) who reported improved agronomic traits such as plant height and number of days to 50% flowering, number of pods per plant and seed weight in soybean seeds treated with gamma rays. Results obtained on number of days to flowering and maturity are in agreement with Mahala *et al*. (1990) who reported that mutagenesis could widen variability to either positive or negative direction which resulted in a sufficient variability in the treated population and this could be utilized for selecting early or late flowering plants. Applying gamma rays at lower doses stimulated early flowering and this is probably due to the fact that biological damage increases with the increase in dose at faster rate than the mutations. This was in tandem with the report of Jan *et al*. (2011) who observed that lower doses of gamma rays significantly improved vegetative traits of *Psoralea corylifolia* L but higher doses significantly reduced same parameters. The good agronomic performance of soybean seeds irradiated with 150 Gy and 250 Gy could probably also be due to suppression of root-knot activities in those treatments. This is because, root-knot nematodes infested plants suffer considerable reduction in their growth and show typical symptoms of root galling, stunting and nutrient deficiency; particularly nitrogen deficiency, and water stress or soil-borne diseases (Hunt *et al*., 2005; Starr and Mercer, 2009; Jones *et al*., 2013) which lead to reduced growth.

Table 2: Root galling index, population of second stage juveniles (J2), reproduction index (RI) and reaction level of soybean seeds infested with *Meloidogyne* spp.

Treatments	[a]Root gall index	[b]J2/ 200 cm^3 soil	[c]RI	[d]Reaction level
0 Gy	4.97a	505 (22.48a)	97.12b	S
150 Gy	0.47c	44.00 (6.67b)	8.46e	VR
200 Gy	2.00b	141.00 (11.90b)	27.12d	SR
250 Gy	1.50b	270.00 (16.45b)	51.92c	S
300 Gy	5.50a	786.00 (28.04a)	151.15a	S
LSD (0.05)	0.89	116.30 (10.78)	11.24	
CV (%)	16.90	18.30 (4.33)		

[a]Root gall index based on 0-10 scale (Bridge & Page, 1980).
[b]Figures in parenthesis are transformed values ($\sqrt{x}$+0.5), where x is mean number of second stage juveniles.
[c]RI: Reproduction index = Final second stage juveniles extracted from the soil/initial J2 in the field × 100 (Taylor, 1967).
[d]Reaction level based on the RI where VR-Very Resistant, MR-Moderately Resistant, SR-Slightly Resistant and S-Susceptible.
Means followed by the same letter are not significantly different (P > 0.05) according to Duncan's multiple range test.

Conclusions

The study found 150 Gy as the optimum dose required for soybean seed treatment to suppress root-knot nematodes population, reduce galling and subsequently increased yield of soybean var Jenjuma. Maturity period was found to be shorter in treated seeds as compared to the unirradiated control. Root-knot nematodes reproduction and population was highly inhibited in soybean seeds treated with 150 Gy gamma radiation. Therefore, seeds of soybean var Jenjuma can be treated with 150 Gy gamma radiations to manage RKN in soybean production.

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