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Impact of Gamma Irradiation on Tomato, and Pepper Growth Parameters, Phytochemical, Nematode Infectivity and detection of DNA Damage by Comet Assay

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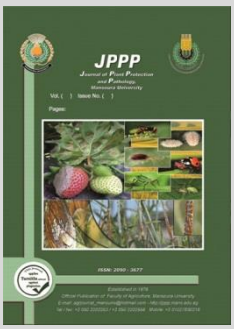

ABSTRACT

Root -knot nematode is a challenge of most vegetable production and there is a critical need to an alternative, effective, safe technique for control. The main objective of this study was to detect the effect of five doses of gamma irradiation on Meloidogyne incognita (Mi) infectivity, host growth, and DNA damage. Three-week-old- seedlings of: tomato, (Solanum lycopersicum), and pepper (Capsicum annum), were irradiated with five doses i.e. 100, 250,500,750, and 1000 Gy and transplanted in 20cm diameter pots in the screen-house. Halves of the replicates/ dose were inoculated with 1000 2IJs, while others were kept as controls. DNA damage was measured by comet assay which performed on the host leaves. Data demonstrated that, all nematode parameters were decreased beginning from the lowest does (100Gy), while, the plant growth parameters were enhanced significantly at 100 and 250Gy even when infected with nematodes and so did the chlorophyll a,b, carotenoids, and total phenols, antioxidative enzymes; Polyphenol oxidase (PPO), peroxidase (POX), Catalase (CAT) and Superoxide dismutase (SOD). Likewise, quantity of DNA damage was represented as mean of % DNA in tail, tail moment, tail length, and olive tail moment which were more pronounced than the control. As a conclusion, gamma irradiation at 100 and 250 Gy could protect host plants of nematode infection and increase the plant growth. Therefore, this approach introduces a promising technique in the integrated pest programs without any suppressive effects on the growth of plant hosts.

Keywords: Gamma Irradiation, Antioxidative Enzymes, Root- Knot Nematodes, DNA damage, comet assay.

INTRODUCTION

Root-knot nematode is an obligate endoparasite which is considered one of the important plant parasites that affects the quality and quantity of the host plant causing disruption in plant vascular system and root galling formation. Meloidogyne can infect most of vegetables, fruits, and ornamental plants all over the world (Perry *et al.*, 2009; and Khalil, 2013) and may cause damage in plant production up to 80% that estimated statistically by US to be $157 billion worldwide (Singh *et al.*, 2015). The ecological and biological persistence ability of this nematode under unfavorable environmental conditions makes their control so difficult. To preserve our environment of chemical hazards, we have to restrict the use of pesticides and find an efficient alternative method to suppress the nematode population. Irradiation is representing a potential application as an alternative to chemicals (Zaka *et al.*, 2004).

Gamma radiation, X-ray, and electron beam are commercially used for sanitizing plants however; the penetrability of gamma irradiation is much higher into materials than the two rest types of irradiations (Hong *et al.*, 2017). Golan and Follett (2017) recommended establishing the irradiation technique in the integrated pest management, that there was no reproductive potential of root-knot nematode, and so there was no galling formation in the irradiated host when compared to control. Irradiation up to 1000 Gy (1 kGy) has been approved of The United States Food and Drug Administration (FDA) for disinfestation and preservation the fresh vegetables and fruits, while 150 Gy was approved dose for the tephritid fruit fly, and 400 Gy for some other insects of Animal and Plant Health Inspection Service (APHIS) (USDA APHIS, 2006). Moreover, the growth of some plant pathogens were inhibited by gamma irradiation, such as fungi (Kim and Yook, 2009; Jeong *et al.*, 2015; Chu *et al.*, 2015). Recently, it is currently permitted by more than 50 countries (Sumira Jan *et al.*, 2013), furthermore FDA approved irradiation of tomato fruits up to 1000 Gy for delaying ripening (FDA, 1986). In addition, the storage period for some vegetables was elongated at the dose of 1 and 2 kGy without any significantly change in the vitamin C content (Kaur *et al.*, 2014). Gamma irradiation could affect physiology, biochemistry and morphology of the plant growth, and the cell contents of some essential compounds such as chlorophyll A, B, carotenoids, and phenolic compound, flavonoid, alkaloid and antioxidant enzyme activity (Kim *et al.*, 2006; Rahimi and Bahrani, 2011), Moreover, the low doses may induce some useful components in plant cells (Mohajer *et al.*, 2014), while the high doses resulting unfavorable compounds (Malencic *et al.*, 2012; Kumar *et al.*, 2013 and Hong *et al.*, 2018).

Over the past decade, comet assay has turn into the standard methods for measurement of DNA damage due to its simplicity, reliability, sensitivity, low cost, and versatility

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(Fabbrizi *et al.* 2021; Cordelli *et al.* 2021). This assay can be used for quarantine applications to control the trade of oranges exposed to electron beam, X-rays and gamma rays, (Yunhee *et al.*, 2018). For the first time, comet assay was able to evaluate the fraction of radiobiologically within an irradiated population of cells; it was used to assess that the rejoining rate of DNA breaks was relatively homogenous (Olive 2009). Thapa (2004) revealed that exceeding 20 Krh/min has a lethal effect on germination and plant growth of pinus. Apparently, these aspects will help in understanding the effectiveness of irradiated plants in nematodes developing. The present study was conducted to investigate effect of gamma irradiation on tomato and pepper growth parameters, phytochemicals, nematode infectivity and DNA damage.

MATERIALS AND METHODS

Materials:

1- Root-knot nematode culture:

The culture of Meloidogyne incognita (Mi) was reared in the screen-house on eggplant (Solanum melongena), in sterile sandy clay soil. The second infective juveniles (2IJs) were extracted from galled roots by washing the infected roots and cutting into small pieces, and then placed in the mist chamber for egg hatching. The 2IJs were collected and refrigerated for the experimental use.

2- The host plant:

Screenhouse experiments were carried out using three-week-old seedlings of tomato (Solanum lycopersicum L. cv. Castel Rock), and pepper (Capsicum annum L.) which were transplanted separately in 20-cm-diam pots filled with sterilized sandy clay soil.

3- Irradiation technique of seedling:

Three-weeks - old plant seedlings of tomato (Castel Rock), and pepper were irradiated by different Gamma irradiation doses viz. 100, 250, 500, 750 and 1000 Gy. by using the unit of Gamma Cell Irradiation (cobalt 60 source), in National Centre for Radiation Research and Technology (NCRRT).

Experimental methods:

1- Effect of gamma irradiation doses on the root knot nematode population and the host plant growth in screen-house:

Fifty of three-weeks-old seedlings of vegetative host plants: tomato, and pepper, were irradiated with ascending doses of gamma rays (100, 250, 500, and 1000 Gy) and transplanted at the same day after irradiation in 20 cm-diam plastic pots filled with sterilized sandy clay soil. Each dose was replicated ten times and divided into two groups; the first group (5 replicates) received 1000 2IJs /pot, while the other group (the other 5 replicates) was left without nematodes. Ten un-irradiated seedlings were considered as control and also divided into two groups; 5 replicates as a positive control (infected with the same inoculum of nematodes), and the other 5 replicates kept as a negative control (without nematode infection). The experiment was terminated after 60 days from nematode infection. At the end of the experiment, the following data were registered.

Nematode parameters

Number of egg masses, galls, and final population of nematode were recorded, and the rate of reproduction was calculated as:

$$Rr = Pf/Pi$$

a) Plant parameters:

Fresh shoot and root length and weight of such plants were measured and recorded.

1- Effect of 5 doses of Gamma irradiation on antioxidant enzymes activity

a- Extraction

The first and second young leaves of tomato and pepper were used for detection of SOD, CAT, POX and PPO antioxidant enzymes. In this regard, 2g of leaves were homogenized with 10 ml of phosphate buffer pH 6.8 (0.1 M), and centrifuged at 2oC for 20 min at 20000 rpm in a refrigerated centrifuge. The clear supernatant was taken as the enzymes source according to (Mukherjee and Choudhuri,1983).

b- Enzymes activity determination

Superoxide dismutase (SOD) activity was determined by Marklund and Marklund (1974) and Kong *et al.* (1999). Catalase (CAT) activity was assayed according to Chen *et al.* (2000). Peroxidase (POX) activity was determined according to Bergmeyer (1974) and Kong *et al.* (1999). Phenol oxidase (PPO) activity was detected by Kar and Mishra (1976); Razinger *et al.* (2007).

2- Effect of 5 doses of Gamma irradiation on photosynthetic pigments

The method was used for the quantitative determination of chlorophyll a, b according to Vernon and Selly (1966), the concentration for carotenoids was determined according to Lichtentahler (1987).

3- Effect of 5 doses of Gamma irradiation on DNA damage by alkaline comet assay:

a-Extraction

Individual tomato and pepper leaf samples were removed from plant and placed in a Petri dish with 0.1 mM ethylenediaminetetraacetic acid (EDTA), Sörensen buffer (50 mM sodium phosphate, pH 6.8, 0.5% dimethyl sulfoxide (DMSO) kept on ice. By a razor blade the leaf tissue was gently sliced and resulting material was repeatedly dipped in the cold Sörensen buffer. The suspension was filtered through a 30 μm disposable filter (Partec; Műnster; Germany) and centrifuged at 550 g for 5 min at 4ºC (Georgieva and Stoilov 2008).

b- Preparation of alkaline Comet assay

The protocol described by Georgieva and Stoilov (2008) with modifications was followed. Microscope slides were coated with 0.5% normal melting agarose and dried at room temperature. Forty μl of the nuclei suspension was mixed with 40 μl of 0.1% low melting agarose, spread on the slide surface and subjected to gel formation for at least 10–15 min on a cooling plate at 4°C. Lysis was carried out in 10 mM Na2EDTA (pH8), 2.5 M NaCl, 1% N-lauroylsarcosine sodium salt, 1% TritonX-100, 10% DMSO, 10 mM Tris-HCl (pH8), for 15 min at 4°C in the dark. Electrophoresis was performed in prepared TAE buffer (pH8) at 0.5, 1, 2 and 5 V/cm for 10 min for leave nuclei. The slides were dehydrated in 96% and 70% ethanol for 5 min and dried at room temperature. By using solution of the fluorescent dye acridine orange (10 μg/ml) the slides were covered. Fluorescence microscope (Zeiss Jenamed-2) coupled with a digital camera (Samsung Digimax V50) was used to the stained slides visualization. In each dose three independent were performed, and 50 comets were analyzed

per point. Damage was detected according to the fragments intensity which migrated during electrophoresis (Parrella *et al.* 2015).

Statistical analysis:

By using analysis of variance procedure proposed by Snedecor and Cochran (1989), the data of all experiments were statistically analyzed. The differences between means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Results

As for infected tomato treatments, data in Table (1) demonstrated that, 100, 250, and 500Gy increased root and shoot length and increased root and shoot weight significantly. Obviously, these plants were able to recover the effect of nematode infection when exposed to the previous doses. Furthermore, doses (750, and 1000 Gy) decreased the plant growth when compared with their controls.

Table 1. Effect of five doses of gamma irradiation on plant growth parameters of tomato infected with Meloidgyne incognita under screen-house conditions.

Irradiation dose	Tomato plant parameters			
	Shoot length	Root length	Shoot weight	Root weight
100 Gy[	40.3 ^{a} ±0.6	28.8 ^{a} ±1.1	11.6 ^{ab} ±0.8	10.0 ^{ab} ±1.0
100 Gy with *M.incognita*	37.0 ^{bc} ±1.0	26.2 ^{bc} ±0.8	11.3 ^{ab} ±0.7	9.5 ^{abc} ±0.9
250 Gy	36.1 ^{bc} ±0.7	28.0 ^{ab} ±1.0	12.0 ^{a} ±0.7	11.0 ^{a} ±0.5
250 Gy with *M.incognita*	34.0 ^{d} ±1.5	26.1 ^{bc} ±0.9	11.0 ^{ab} ±0.8	11.0 ^{a} ±1.0
500 Gy	35.1 ^{cd} ±0.9	25.1 ^{c} ±0.9	11.1 ^{ab} ±0.7	9.1 bc ±0.9
500 Gy with *M.incognita*	30.4 ^{e} ±1.5	20.0 ^{d} ±1.0	9.1 ^{c} ±0.9	8.1 c ±0.9
750 Gy	21.1 ^{g} ±0.9	16.0 e ±1.6	8.0 cd ±1.0	6.0 ^{d} ±1.0
750 Gy with M.incognita	17.0 ^{h} ±1.5	15.0 ^{e} ±1.0	7.1de ±0.6	5.0 d ±1.0
1000 Gy	15.2 h ±1.2	10.1 f ±0.9	6.0 ef ±0.8	3.0 e ±0.5
1000 Gy with *M.incognita*	11.1 j ±0.7	9.2 f ±1.1	5.6 f ±0.6	3.3 e ±1.1
Check (untreated)	37.5 b ±1.8	25.3 c ±1.5	10.4 b ±0.5	9.3 bc ±0.6
Infected check	24.0 f ±1.0	21.7 d ±1.2	8.3 cd ±1.0	10.4 ab ±0.6

At 5% level of significance, the same letter(s) within a column are not significantly different, while different letters had a statistically significant differences.

In case of pepper, Table (2) revealed that, all plant parameters (root and shoot length and root and shoot weight) were increased significantly when exposed to the lowest two levels: 100 and 250 Gy. However, the shoot weight continuously increased when exposed to 500 Gy. On contrary, the high doses decreased the plant growth considerably.

Tables (3 and 4) demonstrated that, all nematode parameters (egg masses, galls, final population, and the rate of reproduction) were decreased significantly in the two host plants comparable to their controls in all gamma doses.

Table 2. Effect of five doses of gamma irradiation on plant growth parameters of pepper infected with Meloidogyne incognita under screen-house conditions.

Irradiation dose	Pepper plant parameters			
	Shoot length	Root length	Shoot weight	Root weight
100 Gy	54.0 a ±3.0	37.0 a ±5.0	15.1 b ±1.4	12.1 bc ±1.1
100 Gy with M.incognita	46.1 c ±1.9	32.0 bc ±2.1	14.1 b ±1.1	11.0 c ±0.8
250 Gy.	51.0 ab ±2.8	37.0 a ±1.5	17.1 a ±0.9	14.1 a ±0.7
250 Gy with M.incognita	42.1 d ±1.7	33.0 bc ±1.8	15.1 b ±0.9	13.1 ab ±1.1
500 Gy	45.0 cd ±3.0	30.1 cd ±1.7	15.0 b ±1.5	11.0 c ±0.4
500 Gy with M.incognita	36.1 e ±1.1	28.1 de ±0.8	14.2 b ±1.1	8.2 d ±1.1
750 Gy	34.1 e ±2.1	26.1 e ±1.1	12.0 c ±0.7	7.0 de ±0.9
750 Gy with M.incognita	27.0 f ±1.5	22.1 f ±0.7	10.1 de ±0.4	6.0 ef ±1.3
1000 Gy	21.0 g ±2.5	18.1 g ±0.9	9.0 e ±1.8	5.1 fg ±1.1
1000 Gy with *M.incognita*	14.0 hi ±1.6	15.1 g ±1.1	5.0 fg ±1.2	4.1 gh ±0.9
Check (untreated)	48.0 bc ± 1.0	35.0 ab ±0.6	14.1 b ±1.3	11.2 c ±1.1
Infected check	37.0 e ±1.6	30.1 cd ±1.6	11.2 cd ±0.5	12.5 abc ±1.2

At 5% level of significance, the same letter(s) within a column are not significantly different, while different letters had a statistically significant differences.

Table 3. Effect of five doses of gamma irradiation on root-knot nematode, Meloidogyne incognita parameters in tomato plants under screen-house conditions.

Irradiation dose	Nematode parameters in tomato plant			
	Egg masses	Galls	Final population (Pf)	Rate of reproduction (Rr)
100 Gy	186.7 b ±11.0	302.3 a ±12.5	2091.0 b ±30.0	2.1 b ±0.03
250 Gy	151.8 c ±8.6	280.7 b ±8.3	1687.3 c ±107.5	1.7 c ±0.11
500 Gy	141.3 c ±7.5	259.0 c ±4.0	1307.7 d ± 12.5	1.3 d ±0.01
750 Gy	123.0 d ± 10.0	214.0 d ±16.0	976.0 e ±14.0	1.0 e ±0.01
1000 Gy	104.3 e ±4.5	192.0 e ±12.0	943.0 e ±41.0	0.9 e ±0.04
Infected check	237.0 a ±6.2	311.3 a ±6.5	2311.7 a ±39.5	2.3 a ±0.09

At 5% level of significance, the same letter(s) within a column are not significantly different, while different letters had a statistically significant differences.

Table 4. Effect of five doses of gamma irradiation on root-knot nematode, *Meloidogyne incognita* parameters in pepper plants under screen-house conditions.

Irradiation dose	Nematode parameters in Pepper plant			
	Egg masses	Galls	Final population (Pf)	Rate of reproduction (Rr)
100 Gy	308.1 b ±7.1	360.1 b ±8.2	2341.0 b ±39.0	2.3 b ±0.04
250 Gy	240.0 c ±7.0	307.0 c ±7.0	1987.0 c ±32.0	2.0 c ±0.03
500 Gy	207.0 d ±7.0	274.0 d ±17.3	1906.3 c ±28.5	1.9 d ±0.03
750 Gy	138.0 e ±5.0	231.0 e ±11.0	1608.0 d ±43.0	1.6 e ±0.04
1000 Gy	109.0 f ±8.0	206.0 f ±10.0	1170.0 e ±70.0	1.2 f ± 0.07
Infected check	354.0 a ±9.0	386.0 a ±8.0	2917.0 a ±97.0	2.9 a ±0.10

At 5% level of significance, the same letter(s) within a column are not significantly different, while different letters had a statistically significant differences.

Data in Figure (1) demonstrated that, the pepper plant cell of Chlorophyll a contents (A), Chlorophyll b (B), Carotenoids (C), and total Phenols (D) were increased significantly in the low doses of irradiation (100, 250, and 500 Gy) as follow: 1.17, 0.84, 0.67 for chlorophyll a, and 0.22, 0.52, and 0.36 for chlorophyll b, while recorded 0.377, 1.00, and 0.56 for carotenoids, whilst the total phenols increased in all the irradiation levels (0.27, 0.34, 0.85, 0.69, and 0.87 except the dose of 1000 Gy which recorded the lowest content 0.26, respectively.

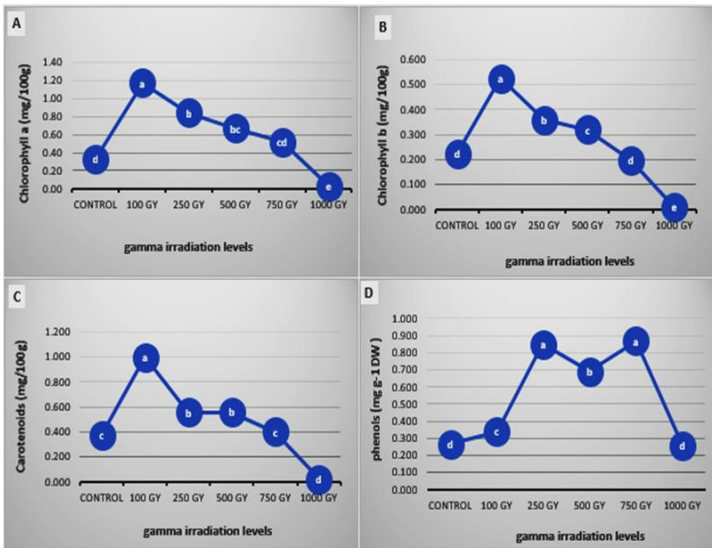

Figure 1. Effect of five doses of gamma irradiation on plant contents of pepper infected with Meloidogyne incognita i.e Chlorophyll a (A), Chlorophyll b (B), Carotenoids (C), and total Phenols (D). Different letters indicate that mean values of treatments are significantly different at P < 0.5.

Tomato contents was more susceptible to Gamma irradiation than those of pepper (Fig. 2). Chlorophyl a (A), Chlorophyll b (B), Carotenoids (C), and total Phenols (D) contents were reduced with the increasing of gamma irradiation doses, and 250 Gy recorded the highest levels of these contents (0.593, 0.292, 0.5200, and 0.596, respectively) comparing with their controls (0.762, 0.376, 0.656 and 0.783 respectively).

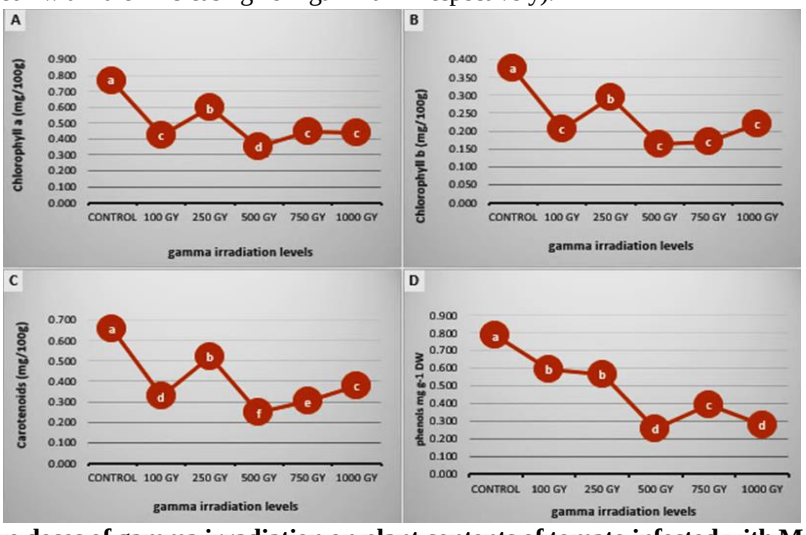

Figure 2. Effect of five doses of gamma irradiation on plant contents of tomato infected with Meloidogyne incognita
Different letters indicate that mean values of treatments are significantly different at P < 0.5.

As shown in Figure 3, the antioxidative enzymes in pepper plants, POX (A), PPO (B),SOD (C), and CAT (D), were highly correlated proportionally to the irradiation doses (100, and 250 Gy) and started to decrease in the high

irradiation doses (500, 750, and 1000 Gy). On the other hand, there was little influence in the case of tomato plants which appeared to be more stability in their enzyme contents (insignificantly differences) (Fig. 4). However, in both plant hosts the most affected enzyme was Catalase enzyme (CAT)

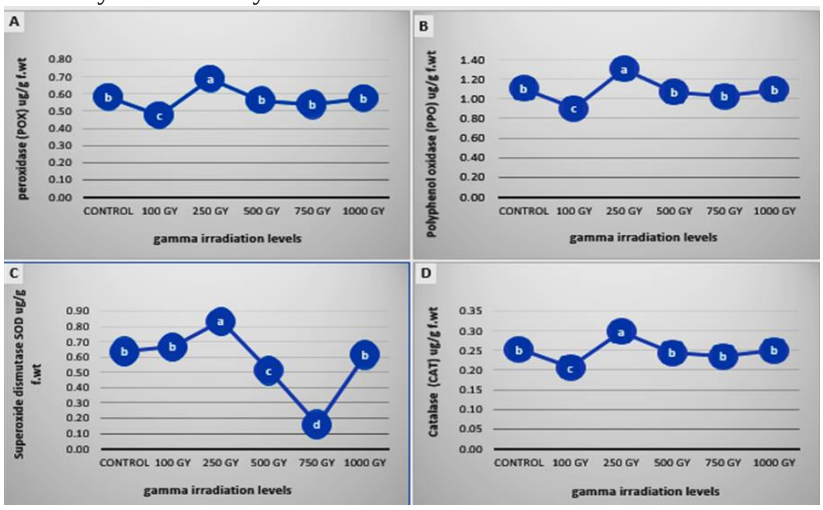

Figure 3. Effect of five doses of gamma irradiation on antioxidative enzymes: POX (A), PPO (B),SOD (C), and CAT (D) in pepper plant infected with Meloidogyne incognita. Different letters indicate that mean values of treatments are significantly different at P < 0.5.

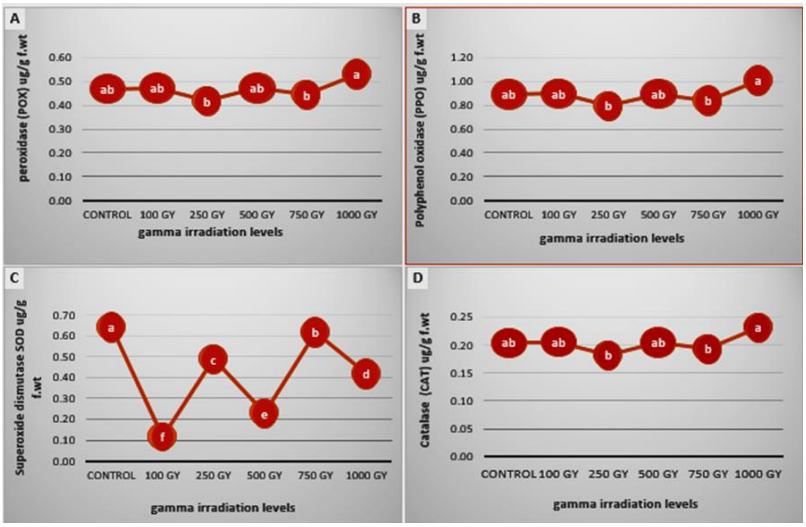

Figure 4. Effect of five doses of gamma irradiation on antioxidative enzymes: POX (A), PPO (B), SOD (C), and CAT (D) in tomato plant infected with Meloidogyne incognita. Different letters indicate that mean values of treatments are significantly different at P < 0.5.

As with DNA damage, tail length, DNA in tail, olive tail moment and tail moment are accurate parameters to detect the effect of gamma irradiation on DNA in the cell. Comet tail was formed because DNA fragments were migrated out of the head and thus DNA in the tail percentage was increased, the DNA damage was comparatively high expressed by % DNA in tail. Generally, the effect on DNA parameters were higher in tomato plants than those in pepper, data in Figures (5, 6) demonstrated that, the effect of irradiation increased proportionally with the doses of irradiation.

In pepper plants (Fig. 5) it was found that, by using 100 Gy, the lowest DNA damage was recorded (7.2 %) and the highest percentage was detected with 1000 Gy (14.6%). On the opposite, the lowest tail length was observed with 1000 Gy (6.56 pixel) and the highest with 100 Gy (8.12 pixel). As with the percentage of DNA in the tail of comet, the lowest percentage (6.41 %) was detected with 750 Gy and the highest percentage was observed with 750 Gy (9.53 %). On the other hand, the lowest tail moment (0.29) was appeared with 250 Gy and the highest one (0.72) was detected with 1000 Gy. For the olive tail moment, the lowest value 0.90 was recorded with 750 Gy while the highest value 1.37 was exhibited with 750 Gy.

In tomato plants, the lowest percentage of DNA damage 7.1 % was recorded with 100 Gy and the highest one 16% was exhibited with 1000 Gy. While the lowest tail length 6.39 pixel was appeared with 250 Gy. For DNA in the tail of comet percentage, the highest percentage 13.8 % was observed with 750 Gy. However, the lowest tail moment 5.59 was appeared with 100 Gy and the highest value (1.01) was recorded with 750 Gy. For the olive tail moment, the lowest value (1.01) was recorded with 500 Gy while the highest one (2.17) was exhibited with 750 Gy. (Fig.6).

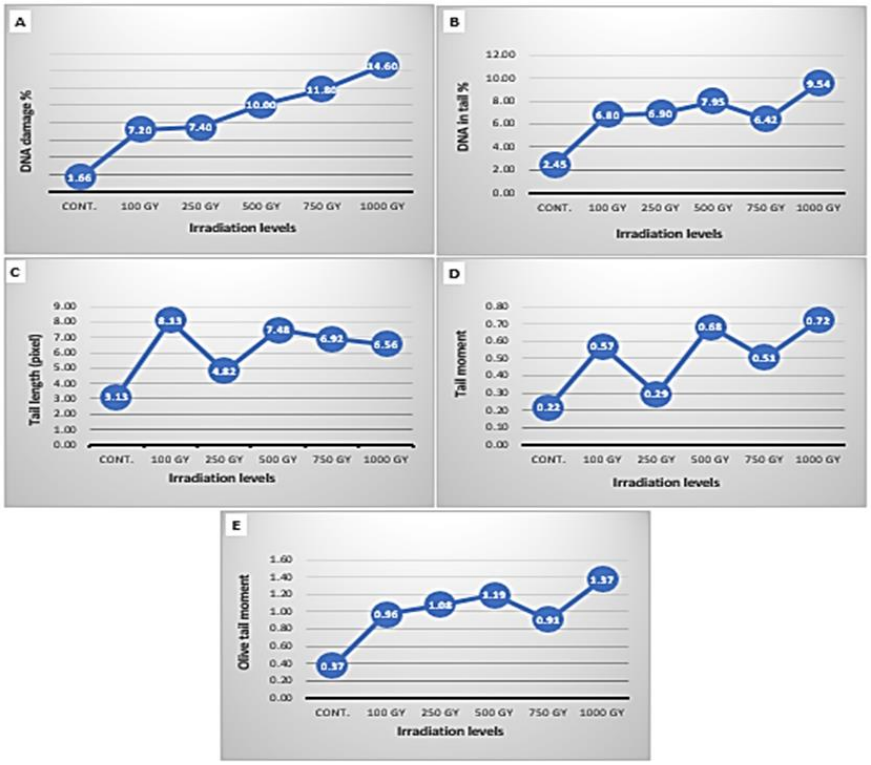

Figure 5. Effect of five doses of gamma irradiation on DNA damage in pepper plants infected with Meloidogyne incognita by comet assay, as well as DNA % (A), DNA in tail (B), tail length(C), tail moment (D) and olive tail moment (E).

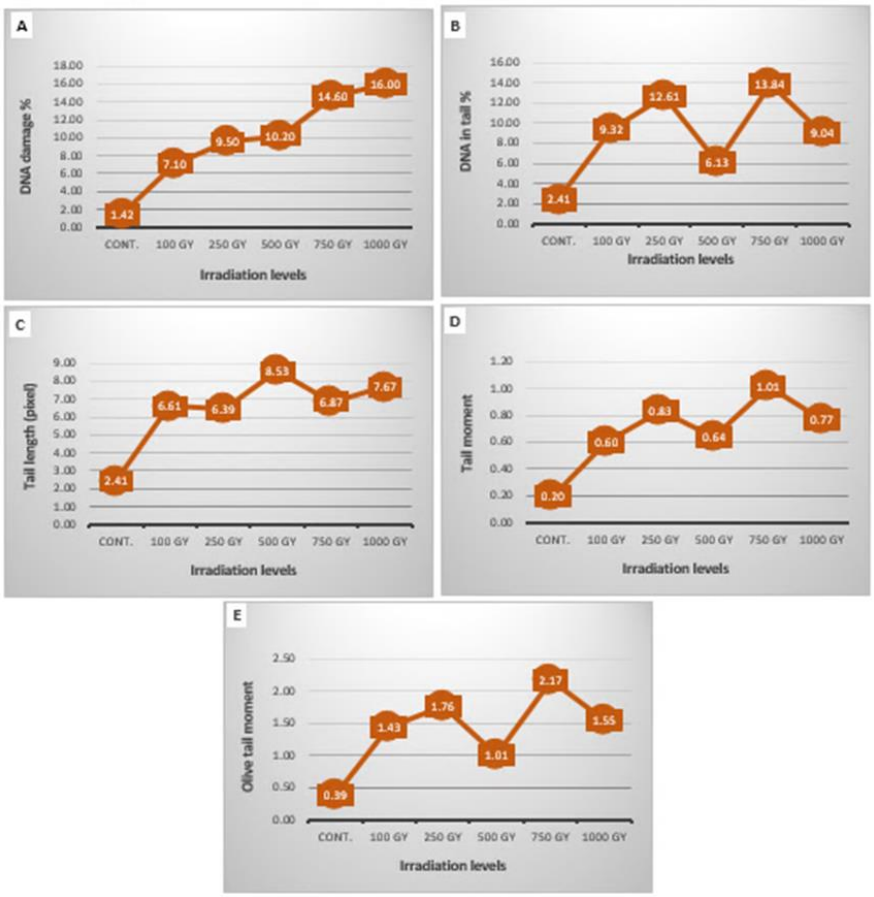

Figure 6. Effect of five doses of gamma irradiation on DNA damage in tomato plants infected with Meloidogyne incognita by comet assay, as well as DNA % (A), DNA in tail (B), tail length(C), tail moment (D) and olive tail moment (E).

Discussio

The present data indicated that, all irradiated plant growth parameters were increased by low doses of gamma rays while the high doses decreased all the plant growth parameters. In this respect, some investigators attributed att gamma rays to accelerate growth rates of tomato plants (Singh and Datta 2010; Hegazi and Hamideldin 2010; Marcu *et al.*, 2013) and induce cytological, physiological, biochemical, genetical, and all of these translated into morphological changes in the plant tissues (Jan *et al.*, 2010), while Thapa (2004); Puzon (2005); Kim *et al.* (2004, 2005); Melki and Marouni, (2009) attributed that to the increasing of physiological activity, by enhancing cell proliferation (Chakravarly and Sen, 2001), increase the plant resistance to some stress factors (Zaka *et al.*, 2002; Lee *et al.*, 2002a; 2002b; 2003), improved the morphological of plant parameters (Maity *et al.*, 2005), and improve the crop yield (Al-Safadi *et al.*, 2000). Additionally, in this line, the exposure of microorganisms to low doses of irradiation directly damages the cellular macromolecules (proteins, ribonucleotides and nucleotides to a lesser extent), or indirectly attacks the target macromolecules through the generation of free oxygen radicals a substantial flux especially hydrogen peroxide and hydroxyl radicals (Smolko and Lombardo 2005).

Our results agreed with those investigators who attributed the side effect of the high doses of irradiation on the plant anatomy, morphology, physiology, and biochemistry, to the disturbing of the plant protein synthesis, hormone levels (Rabie *et al.*, 1996), activity of enzyme (Al-Rumaih and Al-Rumaih, 2008; Stajner *et al.*, 2009; Jan *et al.*, 2011), water and gas exchange in the plant leaves and the level of damage depending on the irradiation dose (Vandenhove *et al.*, 2009; Stoeva and Bineva, 2001), formation of some free radicals, which reduce the cell content of the nucleic acid, and affect the enzyme systems and inhibit some physiological hormones and the synthetic of auxins IAA, and in turn inhibition of the plant growth without clarified mechanisms of the action (Luckey, 1980; Miller, 1987). Also, Gamma irradiation may generate different radicals, which may increase some useful or unfavorable components in plant cells (Mohajer et. al., 2014). These free radicals may change some cellular structures and metabolism in the plant, such as, dilation of membranes, changing the antioxidative system, in addition to increase and accumulation of some phenolic compounds in plant cells (Kwon *et al.*, 2001; Zaka *et al.*, 2002; Kim *et al.*, 2004; Kova´cs and Keresztes, 2002; Kim *et al.*, 2005; and Wi *et al.*, 2005). These conclusive findings may explain the serious nematode reduction in low and high doses in all infected irradiated plants in the present study.

Our results demonstrated that, the content of chlorophyll A, B, and carotenoids increased in the irradiated plants, and this agreed with the observation by Ahmed et. al., (2020) who reported that, these contents were increased in the dried mint leaves with 20 KGy, and in pecan nuts at the same dose of irradiation. However, in high doses of gamma irradiation may cause reactive oxygen species (ROS) accumulation in the cells which lead to the cell organelles damage (Takamiya *et al.*, 2000). Therefore, the free chlorophyll must be degraded so rabidly, and it was noticed that chlorophyll a is more rapid than chlorophyll b (Kariola *et al.*, 2005). Abd- el-Khair *et al.*, 2019; Nunes *et al.* (2019) agreed with the present results in the increase of phenolic contents in the gamma irradiation plant (flavonoids and the aromatic phenols), there phenolic contents may increase the plant resistance to M. incognita (Bajaj and Mahajan, 1977), such phenols will be oxidating by the polyphenol oxidase (PPO) (Mayer and Harel, 1979). The present data approbated that, the levels of antioxidative enzymes such as: SOD, PPO, POD, and CAT were higher in the nematode infected plant than those of uninfected ones. (Ahmed *et al.*, 2020 and Lisiewska *et al.*, 2004) reported the same facts. This may be correlated to the resistance mechanisms of the infected plants (Bajestani, *et al.*, 2019; Khanna *et al.*, 2019; Gupta *et al.*, 2019).

The results of DNA analysis were important in detection the level of damage with five irradiation dosages in tomato and pepper plants. The data showed that, with increasing irradiation dose, the higher numbers of comet types were increased while irradiated samples were exhibited significantly longer DNA comet tail lengths. Our results are in agreement with those of Ptáˇcek et al, (2001) who explained that, gamma irradiation with tobacco seedlings induced a dose dependent increase in somatic mutations. Immediately after irradiation, the increased of somatic mutations were highly correlated with the increased of DNA damage detected by comet assay. While leaves nuclei that isolated after irradiation (24 h) were not significantly litter from the control with tail moment values. These results suggested that, a complete repair of DNA damage were induced by gamma-irradiation and observed by the comet assay, whereas the somatic mutations were increased in relation to dose. With increased gamma irradiation dose, the averaged tail moment values were significantly increased. Gichner and Plewa (1998) and Gichner *et al.*(1999) reported a high correlation between DNA damage as detected by comet assay and somatic mutations in tobacco seedlings treatment with the monofunctional alkylating agents. Yunhee *et al.* (2018) used comet assay as a screening and measurement method for comparing irradiated from non-irradiated foods, and hence could supply a practical basis for irradiated foods quarantine control. Fabbrizi *et al.* (2021) demonstrate that, enzyme-modified neutral comet (EMNC) assay can be used clearly to quantify the levels of complex DNA damage (CDD) in the presence and absence of irradiation, and indeed, could be applied for the assessment of other DNA damage agents for their capability to induce CDD. Moreover, EMNC assay can also be utilized to establish the capacity of populations cells and individual cell to repair CDD. However, the EMNC is an oriental methodology and resource for levels of quantitatively determining CDD following different sources of heavy ions and ionizing radiation, but also in different cell models to appear specific details regarding the CDD repair efficiency.

Beside all information mentioned above, many scientists reported that, the low doses of gamma irradiation may enhance the plant growth by the effect on the plant hormones and the antioxidant enzymes to overpower any stress factors such as plant parasitic nematodes (Moghaddam *et al.*, 2011; Mohajer *et al.*, 2014). The present study fit this finding.

CONCLUSION

The low doses (100, 250, and 500 Gy) of gamma irradiated seedlings may be an effective, safe, and could be used as a promising technique in the integrated pest programs without any suppressive effects on the plant hosts and nematode control.

List Of Abbreviations

Mi = Meloidogyne incognita, POX = peroxidase, PPO = Polyphenol oxidase, SOD = Superoxide dismutase, CAT = Catalase, EMNC = enzyme-modified neutral comet, APHIS = Animal and Plant Health Inspection Service

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تأثير استخدام المعاملة باشعة جاما على نمو شتلات الطماطم والفلفل والمحتوى الكيماوى وعلى والقدرة الامراضية لنيماتودا تعقد الجذور Meloidogyne incognita بالاضافة الى قياس تلف الـ DNA باستخدام تقنيةcomet

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تعتبر نيماتودا تعقد الجذور من أهم اجناس النيماتودا الممرضة للنبات والمسببة لأضرار اقتصادية فادحة لمعظم العوائل النباتية التى تصيبها ونظرا لزيادة الوعى بالاضرار الناجمة عن استخدام المبيدات الكيماوية على الانسان والحيوان والنبات والبيئة المحيطة فانه يعتبر من التحديات الهامة جدا ايجاد وسائل مكافحة بديلة للمكافحة الكيماوية يمكن الاعتماد عليها فى برامج المكافحة للنيماتودا. ونظرا لانتشار نيماتودا تعقد الجذور داخل معظم الاراضى الزراعية فى مصر والعالم وصعوبة الاستفادة من الاشعاع فى التأثير عليها كانت فكرة البحث باستخدام شتلات نباتات مشعة لزراعتها فى الاراضى المصابة طبيعيا ودراسة مدى قدرة نيماتودا تعقد الجذور على اصابتها والتكاثر عليها وأجرى البحث كالتالى: تم تشعيع معاملة نباتات طماطم Solanum lycopersicum وفلفل Capsicum annum بجرعات مختلفة من أشعة جاما وهى ٠, ١٠٠, ٢٥٠, ٥٠٠, and 1000, Gy. وتمت زراعتها فى أصص بلاستيكية داخل صوبة سلكية ثم أضيفت لها عدوى بنيماتودا تعقد الجذور Meloidogyne incognita بتركيز ١٠٠٠ يرقة/ اصيص. فى نهاية التجربة (بعد حوالى ٦٠ يوم) تم تسجيل القياسات النباتية لتحديد مدى تأثير الاشعاع على نمو النباتات، كما تم تسجيل القياسات النيماتودية المختلفة لتحديد تأثير استخدام نباتات مشعة على قدرة النيماتودا على الاصابة والتكاثر على النباتات المشعة. وأخيرا تم عمل تحليل لبعض المركبات الكيميائية Chlorophyll a, b, Carotenoids, and total Phenols وبعض الانزيمات النباتية , the antioxidative enzymes; peroxidase (POX), Polyphenol oxidase (PPO), Superoxide dismutase (SOD), and Catalase (CAT), داخل النباتات المشعة، وأخيرا تم عمل تحليل لل DNA داخل النباتات المشعة لتحديد تأثير الاشعاع عليه باستخدام تحليل الكوميت، وكانت نتائج البحث كالتالى: جميع الجرعات المستخدمة من أشعة جاما كان لها تأثير مثبط لنيماتودا تعقد الجذور من حيث القدرة الامراضية والتكاثرية ولكن الجرعات المنخفضة فقط من أشعة جاما ١٠٠, and 250 Gy. كان لها تأثير محفز لنمو النباتات مع عدم التاثير على محتواها من المركبات والانزيمات سابقة الذكر أو ال DNA داخل النباتات المشعة فى حين كان التركيز ٥٠٠ Gy. غير محفز لنمو النباتات وعلى العكس كان للجرعات المرتفعة من الاشعاع تأثير مثبط لنمو النباتات.اعتمادا على ما سبق، فانه الى حدا ما يمكن التوصية باستخدام بعض العوائل النباتية بعد تعريضها لجرعات منخفضة من أشعة جاما وزراعتها فى الاراضى الموبوءة بنيماتودا تعقد الجذور ضمن برامج المكافحة المتكاملة. ولكن الامر يحتاج الى أجراء المزيد من الابحاث لدراسة تأثير الاشعاع على انتاجية النباتات المشعة وتحديد الجرعة الفعالة تحت ظروف الحقل تجنبا لاى خفض كما ونوعا حتى لا يتأثر المزارع اقتصاديا.