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Effect of Radiation and Chemical Mutagens on Seeds Germination of Black Cumin *(Nigella Sativa* L)

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Abstract *Nigella sativa* L., Rananculaceae, is an important medicinal plant. Seed germination responses to gamma and laser radiation, colchicine and 2, 4 Dinitroaniline chemical mutagens at various doses and concentrations were investigated. Low dose of gamma radiation at 5 Kr stimulated seed germination in dry seeds in which FGP at 83.9% was obtained compare to non-radiated dry seeds at 70.8%. Higher doses of gamma radiation (> 5 Kr) had negative effects of seed germination in terms of FGP, GRI and CGRI. Gamma radiation exhibited more severe effects on soaked seeds than dry seeds. Seeds irradiated with 10 and 45 min He-Ne laser gave the highest FGP at 90.07% and 91.67%, respectively. However, 10 min He-Ne laser significantly decreased GRI and CGRI when compared with control seeds and/or other laser doses. In addition, He-Ne laser irradiation decreased the number of days to GT_{50} when compared to the control seeds. For chemical mutagens, 20 mg/l 2,4Dinitroaniline improved FGP when compared with other treatments. Also, colchicine and 2,4Dinitroaniline concentrations significantly increase CGRI when compared with control. It is interesting to note that GT_{50} was decreased with He-Ne laser radiation and increased with gamma radiation while no significant differences were observed with chemical mutagens.

Keywords: Black cumin, gamma radiation, He-Ne laser, colchicine, 2,4Dinitroaniline, seed germination.

Introduction

For thousands of years, medicinal plants have been the most important source of life saving drugs for the majority of world's population. *Nigella sativa* L. (Rananculaceae), an annual herbaceous plant, is commonly known as black seed or black cumin. It has been traditionally used for centuries in the Middle East, Northern Africa and Asia as a spice and food preservative, as well as a protective and curative for numerous disorders and asthma (Nadkarni, 1976). Black cumin seeds contain fixed oil (30%) as well as volatile oil 0.4 - 0.45%). The volatile oil has been shown to contain 18.4 – 24% thymoquinone

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and 46% many monoterpenes such as p-cymene, and α-pinene (El Tahir *et al.*, 1993). Also, many studies reported *Nigella sativa* as immunomodulator (El-Kadi and Kandil, 1987), anti-inflammatory (Houghton *et al.*, 1995) and anti-tumour agents (El Daly, 1998; Mbarek *et al.*, 2007).

Gamma rays are ionizing radiation having low wavelength with high penetrable power, interact with atoms or molecules to produce free radicals in the cells. The free radicals can damage or modify components of plant cells. It has been reported to affect seed germination, morphology, anatomy, and physiochemical characteristics of plants, depending on irradiation level. These effects include changes in the plant cellular structure and metabolism, e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Kim *et al.*, 2004; Wi *et al.*, 2005). Gamma radiation can be useful for the alteration of physiological characters (Kiong *et al.*, 2008). The irradiation of seeds with high doses of gamma rays disturbs the synthesis of protein, hormone balance, gas-exchange, water exchange and enzyme activity (Hameed *et al.*, 2008).

The laser rays (light amplification by stimulated emission of radiation) were discovered by T.H. Maiman using a flash lamp pumped ruby crystal as the medium (Maiman, 1960). Laser has many types such as Argon laser (Blue) and Cobalt laser (Green). One of those types is Helium Neon laser (He-Ne). It emits a fraction of mille watt (mW) of red light at 632.8 nm. Among various applications of laser, it is used as a biostimulator device in agriculture. The laser light at low intensity produces biostimulation when used on seeds, seedlings and plants (Muszyński and Gadyszewska, 2008; Perveen *et al*., 2010; Hernandez *et al*., 2010). Previous studies showed that seed treated with continuous wave He-Ne laser improved germination and accelerated plant growth (Chen, 2005). Various chemical mutagens are used for improving agriculture crops. Many of these chemicals cause chromosomal aberration effects on plants via reactive free radicals (Yuan and Zhang, 1993). Chemical mutagens are a simple approach to create mutation in plants for their improvement of germination behaviour and other related potential agronomic traits (Roychowdhury and Tah, 2011). Colchicines are polyploidizing and mutagenic agent (Bragal, 1955). Previous studies reported that there were reduction in the germination and survival percentage with increasing colchicines concentrations and treatment duration (Pande and Khetmalas, 2012). The effects of dinitroaniline compounds are similar to the effects of Colchicines (Hess, 1982), although there is evidence that colchicine and dinitroaniline herbicides do not bind at the same molecules (Banjeree *et al.*, 1975). Dinitroaniline herbicides effect on plants by interfering with the normal function of microtubules during cell division (Appleby and Valverde, 1989). It has been reported that dinitroaniline herbicides act on plant tissue by preventing tubulin from polymerizing in to microtubules (Jackson and Stetler, 1973). Therefore, normal separation of chromosomes during mitosis is prevented without microtubules formation. The present study investigated the effects of laser and gamma radiation and colchicine and dinitroaniline chemical mutagens on seed germination of *Nigella sativa* with the aim to achieve rapid, uniform and high seed germination.

Materials and methods

Seed materials

Pure and inbred seeds of *Negella sativa*, local variety were used in this study. Seeds were divided into two parts, one part was soaked for 24 hours in water and the other part was kept dry. Both dry (7.7% humidity) and soaked (50.2% humidity) seeds were packed in paper packets for different treatments.

Treatments

Seeds of *Negella sativa* were treated on October 25th, 2011 with radiation and chemical mutagens at three separate experiments as follow:

First experiment: both dry and soaked seeds were irradiated separately with 6 doses of gamma radiation (0, 5, 10, 20 and 30 KR) to form 10 treatments of dry and soaked seeds. The seeds were irradiated at Anshas Nuclear Reactor for Research, Cairo, Egypt. The source of gamma rays was a cobalt-60 wih dose rate of 3.58 min /100 gray.

Second experiment: both dry and soaked seeds were irradiated separately with 7 doses of He-Ne laser radiation (0, 5, 10, 15, 30, 45 and 60 min) to form 14 treatments of dry and soaked seeds. The seeds were irradiated at National Institute of Laser Enhanced Sciences, Cairo University, Egypt with a power intensity 15 $mWcm^{-2}$ He-Ne laser.

Third experiment: dry seeds were soaked in 0.05 and 0.1% colchicine, 10 and 20 mg/l 2,4Dinitroaniline $(O_2N)_2 C_6H_5N_3O_4$ Seeds soaked in tap water for 24 h were served as control.

Pre sowing seed treatments and culture conditions

Treated seeds with radiation and chemical mutagens were sown in sand soil texture at the experimental farm of El-Kanana Company, Al-Buhira government, Egypt on October 26th, 2011. Every treatment consisted of three replications and each replication was represented by 50 seeds. Seeds were sown in straight lines and irrigated with drip irrigation system (the area between two emitters was 50 cm and every emitter had 2 seeds).

Data collection

Germinated seeds were counted every 48 h. A seed was considered germinated when the tip of the radical had grown free of the seed coat (Auld *et al.,* 1988; Dewir *et al.,* 2011). The following germination parameters were recorded: (a) Germination percentage (GP) = (number of germinated seeds/number of tested seeds) $\times 100$.

(b) Germination rate index (GRI) = [(G1/1) + (G2/2) + (Gx/X)] where, G = germination on each alternate day after placement 1, 2, x = corresponding day of germination (Esechie, 1994).

(c) Corrected germination rate index (CGRI) = (GRI/FGP) $\times 100$ where, FGP = final germination percentage.

(d) GT_{50} = number of days lapsed to reach 50% of FGP (Hsu *et al.,* 1985)

Experimental design and statistical analysis

Experiments of gamma and He-Ne laser radiation were set up in a completely randomized design in split plot with two factors (5 doses of gamma radiation and 6 doses of He-Ne laser radiation × two treatments of dry and soaked seeds) and two-way ANOVA. While, chemical mutagens experiment (colchicines and 2,4Dinitroaniline) was set up in a completely randomized design with one- way ANOVA. Each treatment consisted of three replicates and each replicate was represented by 50 seeds. The mean and ANOVA were calculated using SPSS (version 20) statistical program. The mean separations were carried out using Duncan's multiple range tests (Duncan, 1955) and significance was determined at $p \leq 0.05$.

Results and discussions

Effect of radiation on time-course changes in germination percentage of Negilla sativa seeds

Effect of different doses of gamma radiation on the time-course changes in germination percentage of *Nigella sativa* seeds are presented in Fig. 1. The results showed that low dose of gamma radiation at 5 Kr stimulated seed germination in dry seeds in which FGP at 83.9% was obtained compare to non-radiated dry seeds at 70.8% (Fig. 1 A). Higher doses of gamma radiation (> 5 Kr) had negative effects on seed germination. Germination percentage decreased with increasing gamma radiation doses. Gamma radiation exhibited more severe effects on soaked seeds than dry seeds (Fig 1 B). The lowest germination percentage (6.71 and 0.03%) was obtained on 30 KR of both dry and soaked seeds, respectively. The stimulating effects of gamma ray at low doses on germination were attributed to the activation of RNA synthesis (Kuzin *at al*., 1975) or protein synthesis (Kuzin *et al.*, 1976) which occurred during the early stage of germination after seeds irradiated with 4 KR. On the other hand, high doses of radiation causes chromosome aberration in the cells lead to stop cells division into mitosis and this is the most important cellular event after irritation (Evenari, 1965; Datta *et al.,* 1986). It was also observed that increasing the humidity in seeds increased the negative effect of radiation on seed germination. Reduced germination percentage with increasing doses of gamma irradiation has been reported in *Nigella sativa* (Datta *et al.,* 1986; Kumar and Gupta, 2007). This accordance with Bharathi *et al.* (2013) on *Withania somnifera*, they reported that lower dosage (5 KR) treated seeds gave the highest germination percentage when compared with higher doses.

Effect of different doses of leaser radiation on the time-course changes in germination percentage of *Nigella sativa* seeds are presented in Fig. 2 A, B. For dry seeds, there was a significant increase in germination percentage (90.48 and 91.67%) at 10 and 45 min leaser radiation when compared with control as well as other treatments. The lowest doses (control and 5 min) leaser radiation gave the lowest germination percentage 70.8 and 69.64%, respectively. While in soaked seeds all treatments (except 60 min) had no significant differences in germination percentage when compared with control. Also, in dry seeds, higher dose (60 min) gave the lowest germination percentage (73.21%) when compared with other doses. These results indicated that seed moisture content is a determine factor with radiation on seed germination. Previous study by Abdel-Fatah (2005) mentioned that moisture improved the effect of laser treatments on seed germination. The pre-sowing seed treatment with laser radiation stimulates the physiological and biochemical changes in the seed (Anisimov *et al.,* 1997). The treated seeds with leaser light can be applied to improve seed germination (Koper, 1994). Many researchers reported that the stimulation effects of laser light are visible in sprouting seeds (Toth *et al.,* 1993; Podleoeny and Podleoena, 2004). The laser light has a positive influence on enzyme activity and the concentration of free radicals in seeds (Galova, 1996; Podleoeny, 2000). Also, Sebanek *et al*. (1989) observed increasing in the activity of some phytohormones, especially indole-3-acetic acid (IAA) in the irradiated seeds.

Effect of chemical mutagens on time-course changes in germination percentage of Negilla sativa seeds

The effects of various pre-sowing seed mutagens treatments on the time-course changes in germination percentage of *Nigella sativa* are presented in Fig. 3. The highest germination at 94.44% and 90.0% was observed on control and 20 mg/l 2,4Dinitroaniline treatments, respectively. While the lowest germination at 68.67% and 59.33% was obtained from 0.1% colchicine and 10 mg/l 2,4dinitroaniline, respectively. The results showed that colchicine and 2,4dinitroalnine concentrations have opposite trend on seed germination. Higher doses of mutagens can lead to death of cells (Kleinhof's *et al.*, 1978).

Previous study by Pande and Khetmalas, 2012 reported that germination percentage decreased with increasing concentrations of colchicine. 2,4Dinitroaniline affect the mitosis cell division and accordingly seed germination. The mode of action of 2,4Dinitroaniline is due to its ability to inhibit the polymerization of tubulin into microtubules (Gunning and Hardham, 1982).

Effect of gamma radiation on FGP, GRI, CGRI and GT_{50} of Nigella sativa seeds

The effects of gamma radiation on FGP, GRI, CGRI and GT_{50} percentages of soaked *Nigella sativa* seeds are presented in Table 1. FGP gradually decreased with increasing gamma radiation doses. Control (0 KR) exhibited 82.62% FGP while 3.5% was recorded at the highest dose of gamma rays at 30 KR. Seeds sensitivity to gamma rays may be due to reduced amount of endogenous hormones, especially cytokinins, as a result of break down, or lack of synthesis caused by radiation (Kiong *et al.,* 2008) Also, low dose of gamma irradiation stimulates cell division but high dose inhibits cell division due to free radicals and DNA damage (Zaka *et al.,* 2004). In addition, soaked seeds had a negative effect on germination percentage. The interaction between gamma radiation and soaked seeds showed that the highest FGP 94.44% was observed on 0 KR of soaked seeds. Also, the same doses of gamma radiation on dry seeds gave a higher FGP when compared with soaked seeds. From that, 5KR improved the FGP of dry seeds when compared with control and other doses. The low doses of gamma rays are stimulating enzymatic activation and inducing embryo to increase the rate of cell division, which affects germination (Sjodin, 1962). Also, the stimulation of germination may be due to the activation of RNA and protein synthesis which occurs during the early stages of germination (Aly, 2010). Several workers have studied the effect of gamma rays on seed germination of *Nigella sativa* and noted that the higher exposures were usually inhibitory, whereas lower exposures were sometimes stimulatory (Datta *et al*., 1986; Kumar and Gupta, 2007). In addition, soaking of seeds increases the negative effect of gamma radiation on germination. Similarly founded that, exposure to gamma- radiation retards seed germination but, its effect differs and it more pronounced at higher seed moisture contents (Kumari and Singh, 1996). The reduction in FGP may be attributed to the delay or inhibition of physiological and biological processes necessary for seed germination, which include enzyme activity (Chrispeeds and Varner, 1976), hormonal imbalance (Khan and Al-Quainy, 2009), and the inhibition of mitotic process (Ananthaswamy *et al.,* 1971). Chromosomal variations have been induced by gamma radiation (Riera-Lizarazu *et al.,* 2000) affect bio functions in cell. The germination speed (GRI and CGRI) and GT_{50} of *Negilla sativa* seeds were significant in gamma treatments. The data showed that GRI and CGRI had the same trend and decreased with increasing gamma doses. The maximum GRI and CGRI were recorded in control at 84.43 and 98.79%, respectively, but further increase in radiation doses decreased them. While the effect of soaking seeds on GRI and CGRI was not significant. The interaction between gamma rays and soaked seeds showed that control of soaked seeds (0 KR) gave the highest GRI and CGRI at 110.68 and 115.41%, respectively. In contrast, high doses 20 and 30 KR had the lowest GRI and CGRI in both dry and soaked seeds. The results showed that control seeds reached 50% of its final germination (GT_{50}) in a minimum time (6.07 days). There was liner increase in days to GT_{50} with increase gamma rays doses. Also, soaked seeds decreased the days to GT_{50} (10.12 days) when compared with dry seeds. The interaction between gamma rays and soaked seeds showed that control of soaked seeds had the lowest number of days to GT_{50} (3.81 days) when compared with control of dry seeds and other treatments of dry and soaked seeds. On the other hand, 30 KR of dry seeds increased the number of days to GT_{50} (19.27 days). The effect of irradiation on the germination speed of seeds has been widely studied and various effects were reported according to species, water content in the seed and radiation dose (Apama *et al.*, 2013). The rate, the speed and the percent of germination were found to be markedly reduced in response to high doses radiation (Van and Cherry, 1967). These results take the same trend with Melki and Dahmani (2009) and Akshatha and Chandrashekar (2013) on *Triticum aestivum* and *Pterocarpus santalinus*, respectively. They observed that low doses of gamma rays gave the highest germination speed and percentage.

Effect of laser radiation on FGP, GRI, CGRI and GT_{50} of Nigella sativa seeds

The effect of laser light on FGP, GRI, CGRI and GT_{50} percentages of *Nigella sativa* soaked seeds are presented in Table 2. The highest FGP (90. 07

and 91.67%) was observed in seeds treated with 10 and 45 min of laser light, while the lowest one (73.21%) was observed on 60 min treatment. Soaked seeds gave a significant increase of FGP (89.0%) when compared with dry seeds. The interaction between laser light and soaked seeds showed that laser doses from 0 to 30 min increased FGP of soaked seeds when compared with dry seeds. While the higher laser doses (45 and 60 min) gave the same FGP of soaked and dry seeds, but the lowest FGP (73.21%) was recorded in seeds treated with 60 min laser light of both dry and soaked seeds. Pre-sowing irradiation of seeds with laser light results in a faster uptake of water and achieving the larger mass during seed imbibing (Podleoeny, 2002). Also, amylolitic enzymes were increased in seeds after irradiation of the seeds with laser light improved germination (Podleoeny *et al.*, 2001). Optimal doses of He-Ne laser irradiation may enhance FGP of *Negilla sativa*. Similar results was observed on amaranth plant by Dziwulska-Hunek *et al.* (2013), they mentioned that pre-sowing of seeds with laser light resulted in a significant increase of germination. Also, Muszynski and Gladyszewska (2008) reported that the germination of He-Ne laser irradiated seeds significantly increased as compared with control.

Clear differences in germination speed (GRI and CGRI) and GT_{50} of *Negilla sativa* seeds were observed in laser light treatments. The results showed that laser doses from 5 to 45 min improved GRI and CGRI when compared with control and 60 min laser light treatments. But, 10 min laser light had the highest GRI and CGRI at 113.07 and 124.34%, respectively. In addition, soaked seeds increased GRI and CGRI with 101.08 and 112.94%, respectively when compared with dry seeds. The interaction between laser light and soaked seeds showed that 10 min laser of dry seeds gave the highest GRI and CGRI with 121.39 and 133. 62%, respectively, when compared with other treatments.

On the other hand, control of dry seeds had the lowest GRI and CGRI with 58.19 and 82.19%, respectively. Laser doses from 5 to 30 min decreased GT_{50} when compared with control and other treatments. There was no significant difference between soaked and dry seeds on GT_{50}. The interaction between laser light and soaked seeds showed that 10 min laser light of dry seeds accelerated sprouting of seeds and had the lowest GT_{50} (3.44). In contrast, control of dry seeds increased GT_{50} with 8.34 days. Many researchers mentioned that laser-based pre-treatments of seeds have significant positive effects on a dormancy breaking, germination stimulating, acceleration seed sprouting and improving the quality of sowing material, this focused on vegetable seeds, like mustard seeds (Anghel *et al.*, 2000) and Muszynski and Gladyszewska (2008) on radish seeds. Podleoeny and Podleoena (2004) reported that laser light accelerated seed sprouting. Also, Muszynski and

Gladyszewska (2008) showed that differences between laser light and control seeds during the whole germination process, expressed in the terms of GT_{50} were not significant, where irradiation improved the germination index. Similar findings by Soliman and Harith (2010) showed that irradiation of *Acacia farnesiana* with He-Ne laser at 1.70 W/cm^2 for 9 min gave the highest value of germination percentage and germination speed, as well as, the lowest value of time to germination and germination period.

Effect of colchicine and 2, 4Dinitroaline on FGP, GRI, CGRI and GT_{50} of Nigella sativa seeds

Effect of colchicine and 2,4Dinitroaline mutagens on FGP, GRI, CGRI and GT_{50} percentages of *Nigella sativa* are presented in Table 3. There were significant differences among various treatments on FGP. The highest FGP frequency recorded in control and 20 mg/l 2,4Dinitroaniline at 94.44 and 90%, respectively, whereas 10 mg/l 2,4Dinitroaniline recorded the lowest FGP at 59.33%. GRI showed similar trend to that of FGP. The highest GRI (138.3) was obtained at 20 mg/l 2,4Dinitroaniline while the lowest GRI (88.48) was obtained at 10 mg/l 2,4Dinitroaniline. Although there were no significant differences between colchicine and 2,4Dinitroaline treatments on CGRI, but all mutagens treatments significant increased CGRI when compared with control.

There was no significant difference between mutagens treatments and control on GT_{50}. From that, the above results could be attributed to the effect of mutagens on the meristematic tissues of the seeds. It is known that higher dose of mutagens can lead to death of cells (Kleinhof's *et al*., 1978), physiological and acute chromosomal damage (Singh *et al*., 1997), onset of mitosis (Yadav, 1987), chromosomal aberrations induced enzyme activity such as catalase and lipase and hormonal activity resulted in reduced germination. Disturbance in the formation of enzymes involved in the germination process may be one of the physiological effects caused by colchicine (Roychowdhury and Tah, 2011). The results of the present study were similar to that of Pande and Khetmalas (2012) in which germination percentage of *Stevia rebaudiana* seeds was decreased with increasing concentrations of the colchicines.

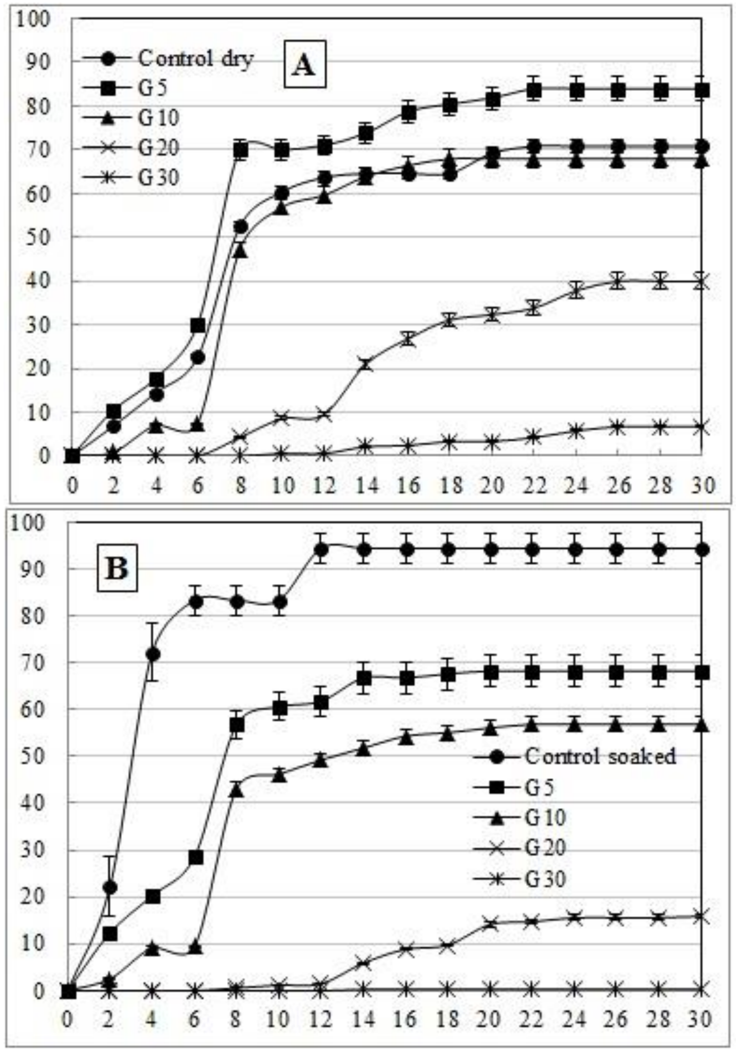

Fig. 1. Time-course changes in germination percentage of *Nigella Sativa* seeds as affected by gamma radiation pre-sowing treatments over 30 days of culture A) Gamma radiation on dry seeds; B) Gamma radiation on soaked seeds.

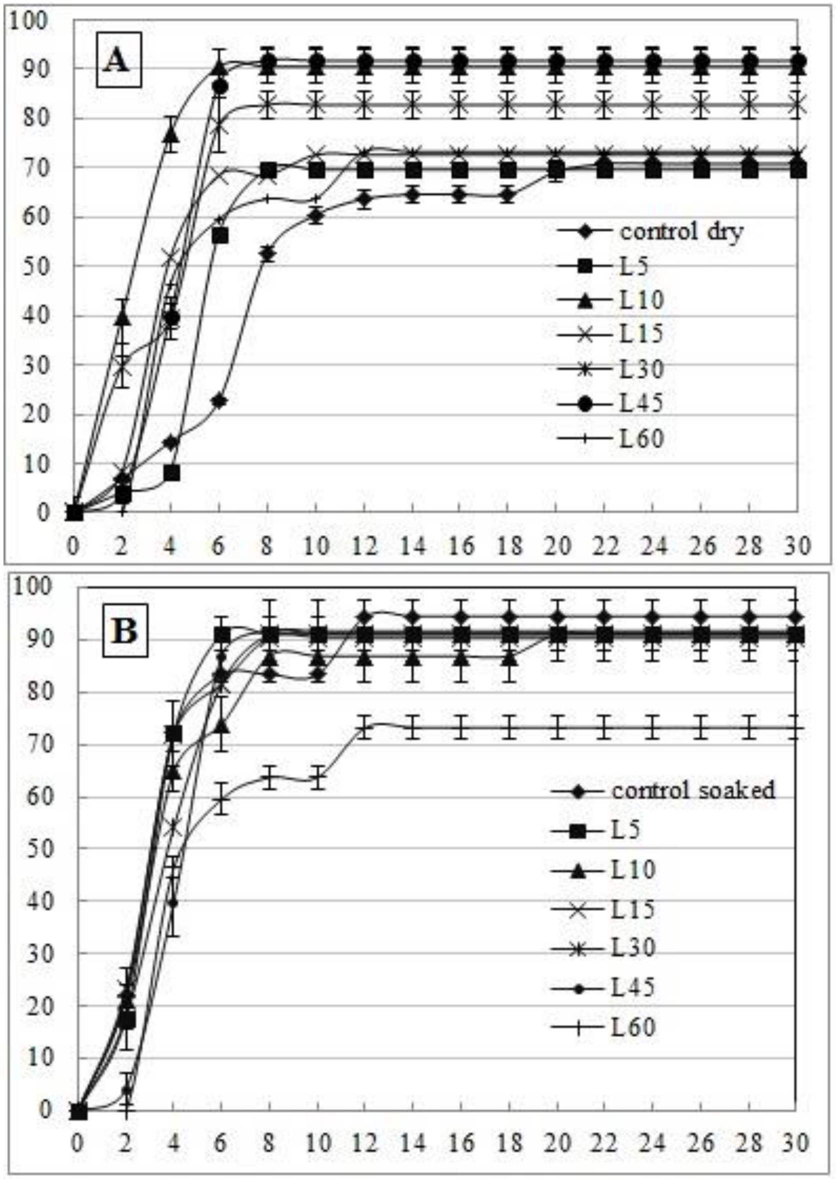

Fig. 2. Time-course changes in germination percentage of *Nigella Sativa* seeds as affected by different laser radiation pre-sowing treatments over 30 days of culture A) Laser radiation on dry seeds; B) Laser radiation on soaked seeds

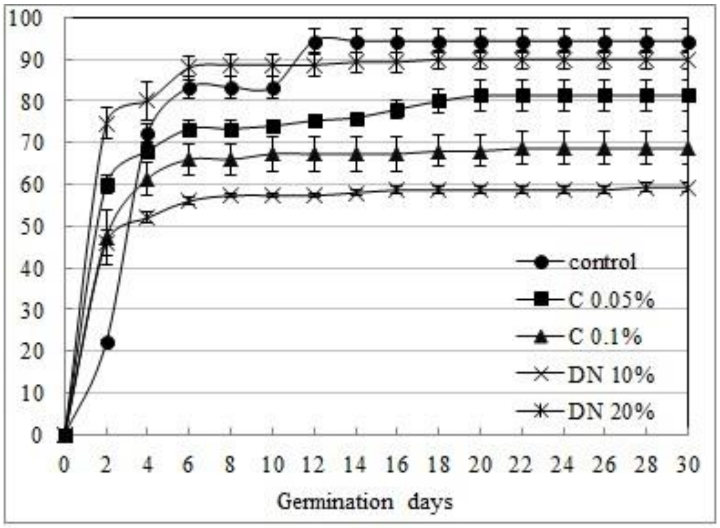

Fig. 3. Time-course changes in germination percentage of *Nigella Sativa* seeds as affected by colchicine (C) and 2,4Dinitroalanine (DN) pre-sowing treatments over 30 days of culture.

Table 1. Effect of pre-sowing gamma radiation seed treatments on final germination percentage (FGP), germination rate index (GRI), corrected germination rate index (CGRI) and number of days lapsed to reach 50% of final germination percentage (GT_{50}) in *Nigella Sativa* after 30 days in culture

Gamma doses (KR)	FGP			GRI			CGRI			GT_{50}		
	Dry	Soak	Mean	Dry	Soak	Mean	Dry	Soak	Mean	Dry	Soak	Mean
0	70.80 c[z]	94.44 a	82.62 a	58.19 bcd	110.68 a	84.43 a	82.19bc	115.41a	98.79 a	8.348 f	3.81 h	6.07 e
5	83.92 b	68.24 c	76.07 b	71.40 b	63.69 bc	67.54 b	85.10bc	93.35 b	89.22 ab	8.08 f	7.06 g	7.57 d
10	67.90 c	56.85 d	62.37 c	49.11 cd	43.25 d	46.18 c	72.33 c	76.08 c	74.20 b	8.89 e	8.74 e	8.81 c
20	39.94 e	14.88 f	27.41 d	16.10 e	5.53 e	10.81 d	40.32 d	34.84 d	37.58 c	15.73 c	17.00 b	16.36 b
30	6.71 g	0.03 h	3.50 e	1.93 e	0.13 e	1.02 d	28.73 d	43.41 d	36.07 c	19.27a	14.00 d	16.63 a
Mean	53.85 a	46.94 b		39.34 a	44.65 a		61.73 a	72.61 a		12.06 a	10.12 b	
Significance	**		**	**		**	**		**	**		**

[Z]Values followed by the same letter in the same column are not significantly different at the 95% level according to Duncan's test, (n = 3).

Table 2. Effect of pre-sowing laser radiation seed treatments on final germination percentage (FGP), germination rate index (GRI), corrected germination rate index (CGRI) and number of days lapsed to reach 50% of final germination percentage (GT_{50}) in *Nigella Sativa* after 30 days in culture

Laser doses min)	FGP			GRI			CGRI			GT_{50}		
	Dry	Soak	Mean	Dry	Soak	Mean	Dry	Soak	Mean	Dry	Soak	Mean
Control	70.80 cd[z]	94.44 a	82.62 ab	58.19 d	110.68 ab	84.43 b	82.19 d	115.41 abc	98.79 d	8.3482 a	3.67 de	6.00 a
5	69.64 d	91.07 a	80.35 ab	66.34 cd	109.75 ab	88.04 ab	95.54 cd	120.30 ab	107.92a bcd	5.8317 b	4.03 de	4.93 bc
10	90.48 a	91.07 a	90.77 a	121.39 a	104.76 bc	113.07 a	133.62 a	115.08 abc	124.34 a	3.4445 e	5.06 bcd	4.25 c
15	72.62 c	90.48 a	81.54 ab	81.92 bcd	110.38 ab	96.14 ab	112.96 bc	119.67 abc	116.31 abc	4.4722 bcde	4.24 cde	4.35 c
30	82.74 b	91.07 a	86.90 ab	99.19 abc	103.68 bc	101.43 ab	120.03 ab	113.93 abc	116.97 ab	4.3468 bcde	4.60 bcde	4.47 c
45	91.67 a	91.67 a	91.67 a	94.60 bcd	94.60 bcd	94.59 ab	103.21 bcd	103.21 bcd	103.21 bcd	5.1310 bcd	5.13 bcd	5.13 abc
60	73.21 c	73.21 c	73.21 b	73.74 bcd	73.74 bcd	73.74 b	99.86 bcd	99.86 bcd	99.85cd	5.7111 bc	5.71 bc	5.71 ab
Mean	78.73 b	89.00 a		85.05 b	101.08a		106.77b	112.49a		5.32 a	4.63 a	
Significant	**		*	*		*	*		*	*		**

[z]Values followed by the same letter in the same column are not significantly different at the 95% level according to Duncan's test, .n=3

Table 3. Effect of pre-sowing colchicine and 2,4Dinitroaniline seed treatments on final germination percentage (FGP), germination rate index (GRI), corrected germination rate index (CGRI) and number of days lapsed to reach 50% of final germination percentage (GT_{50}) in *Nigella Sativa* after 30 days in culture

Treatments	**FGP**	**GRI**	**CGRI**	**GT_{50}**
Control	94.44 a[z]	110.68 ab	115.41 b	3.81
0.05% colchicine	81.33 b	116.71ab	144.00 a	3.74
0.1% colchicine	68.67bc	100.15 b	145.18 a	3.22
10 mg/l 2,4Dinitroaniline	59.33 c	88.48 c	149.08 a	3.18
20 mg/l 2,4Dinitroaniline	90.00 a	138.32 a	153.66 a	2.73
Significant	**	**	**	NS

[z]Values followed by the same letter in the same column are not significantly different at the 95% level according to Duncan's test, (n=3).

Conclusion

Low dose of gamma radiation at 5 Kr stimulated seed germination in dry seeds in which FGP at 83.9% was obtained compare to non-radiated dry seeds at 70.8%. Higher doses of gamma radiation (> 5 Kr) had negative effects of seed germination in terms of FGP, GRI and CGRI. Gamma radiation exhibited more severe effects on soaked seeds than dry seeds. He-Ne laser radiation improved FGP of back cumin seeds at 10 and 45 min treatments. However, the highest GRI and CGRI values were recorded at 10 min treatment. Gamma rays had negative effects of seed germination in terms of FGP, GRI and CGRI. For chemical mutagens, 20 mg/l 2,4Dinitroaniline improved FGP when compared with other treatments. Also, colchicine and 2,4Dinitroaniline concentrations significantly increase CGRI when compared with control. It is interesting to note that GT_{50} was decreased with He-Ne laser radiation and increased with gamma radiation while no significant differences were observed with chemical mutagens.

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