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RESEARCH ARTICLE

IMPACT OF DIFFERENT DOSES OF GAMMA IRRADIATION AND ETHYL METHANE SULPHONATE (EMS) ON PLANT GROWTH AND BULB YIELD OF ONION (*Allium cepa* L.)

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ABSTRACT

This study was conducted at Bangladesh Institute of Nuclear Agriculture (BINA) research farm, Mymensingh during November 2021 to March 2022 to evaluate the effect of gamma irradiation and chemical mutagen on growth and yield of onion. The experiment was laid out in randomized complete block design with three replicates and nine (9) treatments *viz.* four gamma irradiation doses (GD50, GD100, GD150, GD200) and four Ethyl Methane Sulphonate **(**EMS) doses (ED0.1%, ED0.3%, ED0.5%, ED0.7%) and control. The results showed that almost all the parameter studied were significantly influenced by the treatments. The highest germination 90.16%, survivability 94.33%, SCMR value 63.56μmol/ms, Total Soluble Solids (TSS**)** 15.10 %Brix were recorded in T1(GD50) treatment and the maximum mortality 67% in T7(ED0.5%), shoot length 17.18 cm in T5(ED0.1%), root length (3.35 cm) in T5(0.1%), neck diameter 1.06cm in T8 (ED0.7%), bulb diameter 5.36 in T4(GD200), plant height 70 cm in T4(GD200), single bulb weight 75g in T4(GD200) and fresh yield 24t/ha in T5(ED0.1%,). The minimum germination 8.33% in T3(GD150), survivability 36.66% in T8(ED0.07%), mortality 5.66% in T1 (GD50), shoot length 8.26cm in T3(GD150), root length 0.9 cm in T4 , SCMR value 55.26 μmol/ms in T2 (GD100), plant height 51cm in T3(GD150), bulb diameter 3.91 cm in T7(ED0.5) , neck diameter 0.84cm in T6 , single bulb weight 42g in T7 (ED0.5), fresh yield 1.5t/ha in T3(GD150), TSS 10.40 %Brix in T7 (ED0.5%) were observed. Gamma irradiation GD200 (T4) and EMS 0.1% (T5) performed superior considering growth and yield of onion.

KEYWORDS

Mutagen, Gamma irradiation, EMS, TSS, bulb yield, *Allium cepa* L.

1. INTRODUCTION

Onion is the most common spice crop in Bangladesh cuisines and ranked first among all spices in terms of production. Onion (*Allium cepa* L.*)* is a bulbous herb, of the family Alliaceae, originated in Central Asia and grown throughout the world. According to the DAE, the annual demand of onion is around 24 lakh tonnes. Onion production in Bangladesh was 23 lakh 30 thousand tonnes. Every year, Bangladesh has to import a huge amount of onion from neighbouring and other countries to meet up its demand. Imported 8 lakh tonnes onion to meet the demand this year (Bangladesh post, 2021). The rest of the demand was meet through imports from Egypt, Turkey, Myanmar, China, Pakistan and India. Among the world's largest ten onion producing countries, five are from Asia, namely, China, India, Iran, Pakistan and Republic of Korea.

China and India rank top in the list of producers and exporters with China being the top producer and India as the lead trader. Low yield, a lack of quality seeds and inadequate acreage are the main reasons behind the shortfall in domestic production. The basic and foremost reasons of onion are the biennial habit, longer time required for breeding work, maintaining genetic uniformity is difficult due to highly heterogenous nature, highly cross pollinating nature and high inbreeding depression (Lawande et al., 2009). But there is a great gap between demand and supply and to bridge this gap, there is a need to enhance the genetic variability of onion. The genetic diversity through hybridization and traditional method is difficult due to flower morphology, highly cross pollination nature etc.

Therefore, induction of mutation could be a better option to create heritable variability in the form of desirable mutants having particular trait of interest. The current study is therefore undertaken to find out the following objectives. To compare the effective gamma irradiation doses and chemical mutagen doses on growth and yield of onion as well as to find out the effective doses for mutation induction and to produce M1 generation of onion for advancement generation.

2. MATERIALS AND METHODS

The research work was carried out to study the effect of the different doses of gamma irradiation and ethyl methane sulphonate (EMS) on the growth and yield of onion. The experiment was conducted at the Bangladesh Institute of Nuclear Agriculture (BINA) research farm, Mymensingh during 15 November 2021 to 25 March 2022. The experimental area was the subtropical climate which is characterized by heavy rainfall, high humidity, high temperature and relatively long day during the period from April to September and scanty rainfall during the rest period of the year (October to March). The soil of the experimental area was silty loam in texture. The onion seed (cv. Sukhsagor) was used for conducting this experiment that collected from Upazilla Agriculture Office, Mujibnagor, Meherpur District during September 2021. The experiment was laid out in the randomized complete block design with three replications. Thus, the total numbers of plot were 27 with three blocks. The plot size was 3m ×

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1.2m. The blocks were placed 1m apart.

The experiment was conducted with following treatments: T1 (GD50)= 50 Gray; T2 (GD100) = 100 Gray; T3(GD150)= 150 Gray; T4 (GD200) = 200 Gray T5(ED0.1%)= 0.1%EMS; T6(ED0.3%) = 0.3%EMS; T7(ED0.5%)=0.5%EMS; T8(ED0.7% =0.7%EMS; T9= Control(Unirradiated). The seed material was irradiated with different doses of gamma irradiation at the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. For gamma irradiation treatment, the well developed 1000 seeds were selected and subjected for each four treatments to gamma irradiation doses of 50, 100, 150 and 200 Gy of gamma rays in a CO60 gamma source at room temperature (25 ^{0}C). For (EMS) treatment, 1000 seeds were presoaked with distilled water in glass beaker for each four treatments for 16 hours under continuous aeration before the chemical treatment. The seeds were removed from beaker and adsorbed water was removed by pressing under folds of blotting paper.

The presoaked seeds were immediately transferred to freshly prepared 0.1%, 0.3%, 0.5%, 0.7% aqueous solution of EMS in distilled water for 3 hours with regular shaking. The control seeds were soaked in distilled water and were given the same environment as the chemical treatment. The treated seeds were removed from the solution and thoroughly washed in running tap water for 30 minutes to leach out the residual chemical and then were dried on blotting paper. Healthy, well matured and untreated seeds were used as control. The treated seeds were sown on 15 November on the same day in well prepared nursery bed, Horticulture Division, BINA, Mymensingh. The age of seedlings 28 days. In order to grow the M1 generation, the treated seeds along with control were sown in the field in randomized block design with three replications. The onion seedlings were transplanted on 13 December 2021. About one third of the top of the seedlings were cut with proper knife and avoid over aged seedlings. Spacing for seedling transplanting: 15 cm x 20cm.

The treated and control populations were maintained under uniform climatic and agronomic conditions till the harvesting of bulbs. Raising of M1 regeneration, the life cycle of the onion is completing in two phases i) Bulb production and ii) seed production. For bulb production seeds were sown and for seed production bulbs were used for regeneration. The organic fertilizer was applied before land preparation almost 15 days ago of transplanting. Half of all inorganic fertilizer was applied during land preparation; then after 25 DAP. The rest of the fertilizers were applied after 45 DAP. The following intercultural operations were done during the period of the field experiment such as weeding, drainage, insects and diseases control, harvesting. Data were collected on seed germination (%), plant survival (%), mortality (%), plant height, seedling shoot length, seedling root length, number of leaves, bulb diameter, neck diameter, single bulb weight, fresh yield of bulb, chlorophyll content, TSS (Total Soluble Solids).

3. Results and Discussion

Results showed that the germination percentage was higher at lower doses of treatment and germination percentage was lower at higher dosesof irradiation and the difference among the treatments was significant at 1% level of probability (Table 1). The germination percentage of seeds was observed maximum (91%) in T6(ED0..3%) followed by 90.16% and 86.33% on T1 (GD50) and T5(ED0.1%) treatment and minimum seed germination percentage was observed 8.33% in T3(GD150) treatment. The germination percentage was higher at lower doses of treatment compared with T9 (control) that was 59.76% (Table 1) The reduction in germination percentage due to gamma rays may be attributed to either a drop in the auxin level (Gordon and Webber, 1955) or chromosomal aberrations as reported by Reed (1959) and Sparrow (1961). Shah *et al*. (2008) opined that processes like auxin destruction, changes of the ascorbic acid content, physiological and biochemical disturbances could induce the inhibition of plant germination and development.

The survivability was higher at lower doses of treatment compared with control in both gamma ray and EMS treatments. The difference was significant at 1% level of probability (Table 1). The highest survivability percentage was recorded 94.33% in T1 (GD50) followed by 81.66% and 78.33% in T2 (GD100) and T4 (GD200) and the lowest survivability was observed 36.66% in T8(ED0.7%) followed by 43.33% in T6(ED 0.3%) and 46.33% in T5(ED0.1%) that compared with 79.00% in T9 (control). The survivability was higher at lower doses of treatment compared with control in both gamma ray and EMS treatments. This results supported with stated that the percent reduction in survival rate over control was found to be lesser in EMS treatments (1.04 to 4.16 per cent) compared to gamma ray treatments (2.33 to 39.76 per cent) indicating the property of EMS mutagen as a point mutant that produces less biological damage (Talebi et al., 2012). Increasing frequency of chromosomal harm with increasing radiation dose may be responsible for reduction in plant survival. The reduction in plant survival due to the mutagenic treatments has also been reported in Dianthus, Horsegram, Ashwagandha, Pigeon pea, Pearl Millet, Sesame and Okra (Chowdhury et al., 2012; Bolbhat sadashiv et al., 2012; Bharathi et al., 2013; Ariraman et al., 2014; Ambli et al., 2014; Ramadoss et al., 2014; Baghery et al., 2016).

The difference among the treatments was a significant at 1% level of probability (table 1) on the effect of Gamma Radiation and Ethyl methan sulphonate on mortality percentage (%). The mortality percentage was recorded highest 67% in T7(ED0.5%) followed by 53.33%, 29.33%, 21.66% in T5(ED0.1%), T3(GD150) T4 (GD200) treatment and lowest was found 5.66% in T1 (GD50) followed by 1.33 in T2 (GD100) treatments that compared with 21% in T9 (control). The mortality was higher at higher doses of treatment compared with control (Table 1). The seedling mortality was reported to be due to the decline of assimilation mechanism, inhibition of auxin synthesis, inhibition of mitosis and chromosome damage (Quastler and Baer, 1950; Skoog, 1935; Gunkel and Sparrow, 1961). The LD50 value varies according to crop species, varieties, seeds or other planting materials, nature of treatment, method of raising, climate, cultural practices and other parameters (Singh, 1994).

Table 1: Effect of different levels of Gamma irradiation and Ethyl Methane Sulphonate on germination, survivability and mortality after sowing of onion seed

Treatments	Germination (%)	Survivability (%)	Mortality (%)
T1	90.16	94.33	5.66
T2	34.00	81.66	18.33
T3	8.33	70.66	29.33
T4	32.66	78.33	21.66
T5	86.33	46.66	53.33
T6	91.00	43.33	56.66
T7	63.00	33.00	67.00
T8	31.33	36.66	53.33
T9	59.76	79.00	21.00
LSD (0.01)	2.36	7.56	7.56
Level of significance	**	**	**

**= significant at 1% level of probability, T_1= GD_{50}, T_2= GD_{100}, T_3= GD_{150}, T_4= GD_{200}, T_5= ED $_{0..1\%}$, T_6= ED $_{0.3\%}$ T_7=$ED_{0.5\%}$, T_8= $ED_{0.7\%}$, T_9= Control. GD= Doses of Gamma Radiation, ED= Doses of Ethyl methan sulphonate

Among the irradiated plants highest shoot length was noticed 17.18 cm in T5(ED0.1%) followed by 15.05 cm 14.05cm and 10.62cm in T6 (ED0.3%), T1(GD50) and (ED0.5%) treatments, the minimum shoot length was observed 8.26cm in T3(GD150) and 13.32 cm was recorded in T9 (unirradiated control plants) (Table 2). It was observed that shoot length tended to decrease progressively with increasing dose of irradiation with significant disparity among the doses (Table 2).). Among the difference of the treatments the probability was significant at 1% level of probability (Table 2). Decrease in the shoot length with an increase in the irradiation dose might be attributed to the biochemical and physiological changes induced by gamma irradiation (Melki and Marouani, 2009). The chromosomal damage and inhibition of cell division are the chief causes of reduced seedling growth. The inhibition in seedling growth might be due to the gross injury caused at cellular level either due to gene controlled biochemical processes or acute chromosomal aberrations or both. A similar line of research work had been documented in Costus, in ginger, in yams (Gupta et al., 1982; Giridharan, 1984; Nwachukwu et al., 1990).

The difference was significant at 1% level of probability. Among the treatments, the superior root length was recorded 3.35 cm in T5 treatments while minimum root length was observed 0.9 cm in T4 treatments. However, in the T9 (control) root length was recorded (3.06 cm) (Table 2). The stimulatory effect on root length was observed in the lower doses of gamma rays. The stimulatory effect on root length was observed in the lower doses of gamma rays (Table 2). The hypothetic origin of these stimulations by irradiation was due to the cell division rates as well as an activation of growth hormone, such as auxin (Zaka et al., 2004.

Among the difference of the treatments the probability was non significant. The Number of leaves ranged 7.66 (T1) to 7.4 (T2) in Gamma ray treatment and in EMS treatments, the Number of leaves ranged from 7.66 (T5) to 6.66 (T6) that compared with T9 (control) (Table 2). These results coincided with the findings of in okra (Jagajanantham et al., 2012). The decrease in the number of leaves might be attributed to the changes caused by gamma irradiation in plant morphology and function, as affected by various dosage and duration of exposure time (Piri et al., 2011). It is also predicted that enzymes essential for leaf initiation at higher intensities of gamma irradiation might have stopped and blocked the cell division by decreasing the rate of physiological processes (Akhtar, 2014).

Table 2: Effect of different levels of Gamma irradiation and Ethyl Methane Sulphonate on seedling shoot length, seedling root length, and no. of leaves after 28 days of seed sowing

Treatments	Seedling shoot length (cm)	Seedling root length (cm)	No. of leaves/plant
T1	14.05	1.95	7.66
T2	13.34	2.34	7.4
T3	8.26	1.89	7.33
T4	10.46	0.9	7.66
T5	17.18	3.35	7.66
T6	15.05	1.99	7.33
T7	10.62	2.44	6.66
T8	10.02	2.46	7.13
T9	13.32	3.06	8
LSD (0.01)	4.51	1.86	2.01
Level of significance	**	**	NS

**= significant at 1% level of probability; NS = Non-significant, T_1= GD_{50}, T_2= GD_{100}, T_3= GD_{150}, T_4= GD_{200}, T_5= ED $_{0.1\%}$, T_6= ED $_{0.3\%}$, T_7=$ED_{0.5\%}$, T_8= $ED_{0.7\%}$, T $_9$= Control. GD= Doses of Gamma Radiation, ED= Doses of Ethyl methansulphonate

The difference among the treatments was statistically nonsignificant. Among the treatments, the highest SCMR value 63.56 µmol/ms in $T_1(GD_{50})$ followed by 62.77 µmol/ms, 60.98 µmol/ms, 57.23 µmol/ms in $T_7(ED_{0.5\%})$, $T_8(ED_{0.7\%})$ and $T_3(GD_{150})$ where the lowest chlorophyll content was observed in T_2 (GD_{100}) (Table 3) compared to the non-irradiated treatment 60.98 µmol/ms in control (T $_9$). Among the difference of the treatments was statistically non-significant (Table 3). This result supported with Kimetal opined that the significant increase in the chlorophyll content can be correlated with stimulated growth at low doses of irradiation (Kimetal, 2000). A group researcher reported that reduction in chlorophyll was due to a more selective destruction of chlorophyll biosynthesis or degradation of chlorophyll precursors (Kiong et al., 2008).

Among the treatments T_8 $(ED_{0.07\%})$ showed highest neck diameter 1.06 cm followed by 1.03 cm, 0.97cm, 0.98 cm in T_2 (GD_{100}), $T_1(GD_{50})$ and T_4 (GD_{200}) and lowest neck diameter treatment was found 0.84 cm in $T_7(ED_{0.5\%})$ (Table 3) that was similar results 0.84 cm in $T_6(ED_{0.3\%})$ compared to the non-irradiated 0.96cm in control (T_9). Among the difference of the treatments was statistically non-significant on (Table 3) the effect of gamma irradiation doses and ethyl methane sulphurated on neck diameter/neck thickness. Although this parameter is one of the most important parameters when we consider the shelf life of onion. In general, it being consider that the less neck thickness the more shelf life as large neck thickness provide more surface area to microbes for infection. Bulb with thin necks store longer than bulb with thick necks (Gautam et al., 2006).

Among the treatments T_8 $(ED_{0.7\%})$ showed highest neck diameter 1.06cm followed by 1.03 cm, 0.97cm, 0.98 cm in T_2 (GD_{100}), $T_1(GD_{50})$ and T_4 (GD_{200}) and lowest neck diameter treatment was found 0.84 cm in $T_7(ED_{0.5})$ (Table 3) that was similar results 0.84 cm in $T_6(ED_{0.3\%})$ compared to the non-irradiated 0.96cm in control (T $_9$). The difference among the treatments was statistically non-significant (Appendix4). The most important indicators of productivity are the diameter of onion bulbs (Table 3). The difference among the treatments was statistically significant at 5% level of probability. The mortality was higher at higher doses of treatment. So the bulb diameter was increased at higher doses of the treatment due to reduction in plant population probably attributed to more nutrients, space, moisture availability resulting in enlargement of their bulb size. Similarly, high plant density implies closer spacing and ultimate reduction in space available per plant and then the tendency is real that bulb expansion might be limited due to smaller space for bulbing. The findings is in line with (Nigulle and Biswas 2017) who found the highest bulb diameter from intra row spacing.

Table 3: Effect of different levels of Gamma irradiation and Ethyl Methane Sulphonate on chlorophyll content, neck diameter and bulb diameter at mature stage

Treatments	Chlorophyll content (µmol/ms)	Neck diameter (cm)	Bulb diameter (cm)
T1	63.56	0.97	4.82
T2	55.26	1.03	4.97
T3	57.23	0.98	4.74
T4	60.18	0.97	5.36
T5	62.43	0.94	4.54
T6	59.3	0.84	4.12
T7	62.77	0.84	3.91
T8	60.98	1.06	4.73
T9	60.98	0.96	4.73
LSD 0.05	9.17	0.23	0.74
Level of significance	NS	NS	*

*= significant at 5% level of probability; NS = Non significant, T_1= GD_{50}, T_2= GD_{100}, T_3= GD_{150}, T_4= GD_{200}, T_5= ED $_{0.1\%}$, T_6= ED $_{0.3\%}$, T_7=$ED_{0.5\%}$, T_8= $ED_{0.7\%}$, T $_9$= Control. GD= Doses of Gamma Radiation, ED= Doses of Ethyl methansulphonate

The difference among the treatments was significant at 5% level of probability. At 80 DAT, the plant height was recorded maximum 70 cm in T4(GD200) treatment followed by 59 cm and 56 cm in T2(GD100) and T8(ED0.7%) and the minimum plant height was recorded 51cm in T3(GD150) treatment whereas the control plant height was recorded 65 cm in control(T9) (Figure 1). Plant height was also found to be significantly reduced in higher doses of physical and chemical mutagenic treatments (Figure 1). The results disagree with who reported decrease in shoot length at high dose (Toker et al., 2005). Low doses of ionizing radiation induce hermetic or hormesis effects which alter photosynthesis, stimulation of growth and other physiological processes. However, disparity in the effects of gamma radiation on plant growth was reported by (Melki, Marouani 2010).

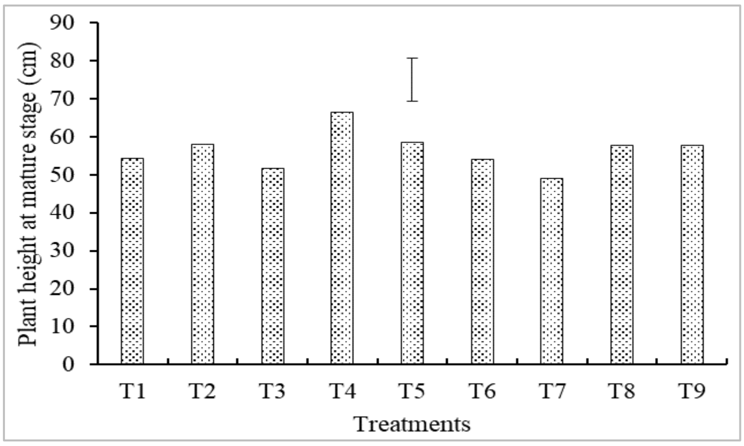

Figure 1: Effect of different levels of Gamma irradiation and Ethyl Methane Sulphonate on plant height at mature stage (cm)

Vertical Bar represents LSD at 5% level of probability. T1= GD50, T2= GD100, T3= GD150, T4= GD200, T5= ED 0.1%, T6= ED 0.3% T7=ED0.5%, T8=ED0.7%, T 9= Control. GD= Doses of Gamma Radiation, ED= Doses of Ethyl methan sulphonate

The difference among the treatments was statistically significant at 5% level of probability. The most important indicators of productivity are weight of onion bulbs. Among the treatment, highest single bulb weight 75g was recorded in T4(GD200) followed by 69g in T2(GD100) treatment where the lowest was observed 42g in T7 (ED0.5%) followed by 52g in T5(ED0.1%) compared to the non-irradiated 60.98 g in T9(control) (Figure 2).

The most important indicators of productivity are weight of onion bulbs (Figure 2). The difference among the treatments was statistically significant at 5% level of probability). This results coincided with stated that pre-sowing seed treatment with DMS also stimulated the growth and development of *Calendula officinalis* L. plants (Khazieva et al., 2016). They exceeded the control variants in biometric parameters, raw material and seed yields.

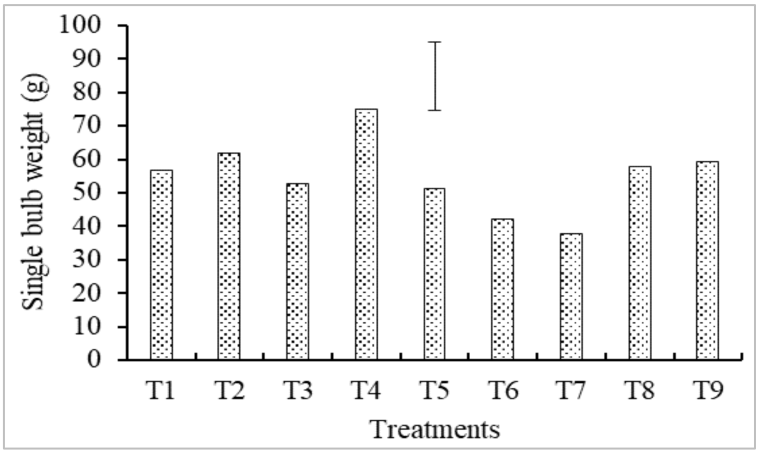

Figure 2: Effect of different levels of Gamma irradiation and Ethyl Methane Sulphonate on single bulb weight (g).

Vertical Bar represents LSD at 5% level of probability. T1= GD50, T2= GD100, T3= GD150, T4= GD200, T5= ED 0.1%, T6= ED 0.3% , T7= ED0.5%, T8= ED0.7%, T 9= Control. GD= Doses of Gamma Radiation, ED= Doses of Ethyl methan sulphonate

The difference was statistically significant at 5% level of probability. So the maximum Yield fresh weight was observed 24t/ha in T5(ED0.1%,) followed by 20 t/ha and 18t/ha in T1(GD50) and T2(GD100) treatment. Lowest Yield fresh weight 1.5t/ha was found in T3(GD150) treatment where as compared with 16t/ha in T9(control) (Figure 3). The difference

was statistically significant at 5% level of probability (Figure 2). The yield was decreased as the dose of gamma rays and EMS increased. The reason for the increased yield in lower concentrations may be attributed to the enhancing effect and growth regulatory effect of mutagen. The Similar line of research work of reduced yield by increased doses of gamma rays and EMS was also reported in ginger, in turmeric and in okra (Jayachandran, 1989; Neopaney, 1994, 2006; Jadhav et al., 2013).

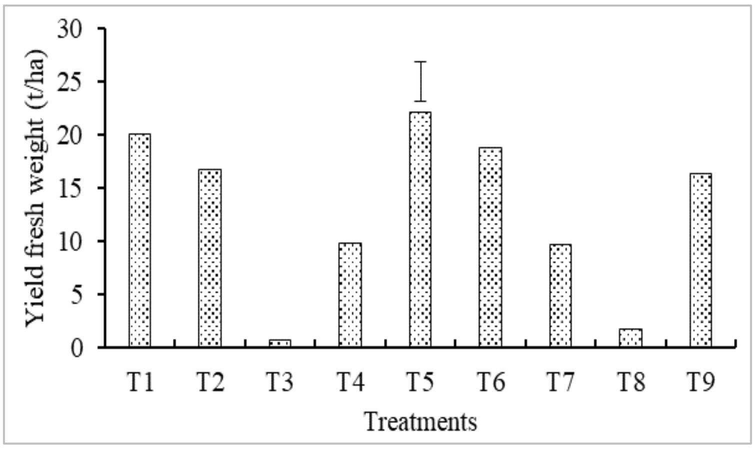

Figure 3: Effect of different levels of Gamma irradiation and Ethyl Methane Sulphonate on yield fresh weight (t/ha)

Vertical bar represents LSD at 5% level of probability, T1= GD50, T2= GD100, T3= GD150, T4= GD200, T5= ED 0.1%, T6= ED 0.3% T7= ED0.5%; T8= ED0.7%, T 9= Control; GD= Doses of Gamma Radiation, ED= Doses of Ethyl methan sulphonate

The TSS was higher at lower doses of treatment compared with control. Among the treatments, Total Soluble Solid was recorded maximum 15.10 (%Brix) in T1(GD50) treatment and lowest TSS was recorded 10.40 (%Brix) in T7 (ED0.5) treatment where as compared with 10.50 (%Brix) T9(control) (Figure 3). The difference was statistically significant at 5% level of probability (Figure 3). The TSS was higher at lower doses of treatment compared with control. Total Soluble Solids (TSS) on onion gradually increased up to 40 days and then found to be decreased until termination of storage period for all treatments (Figure 4). However, significant ($p < 0.05$) difference in total soluble solids (TSS) was found up to 125 days from harvesting to storage period. But this result disagreed with the finding of Curzio and Croci that the radio inhibition process does not adversely affect the levels of ascorbic acid content in onion bulb (Curzio and Croci, 1988).

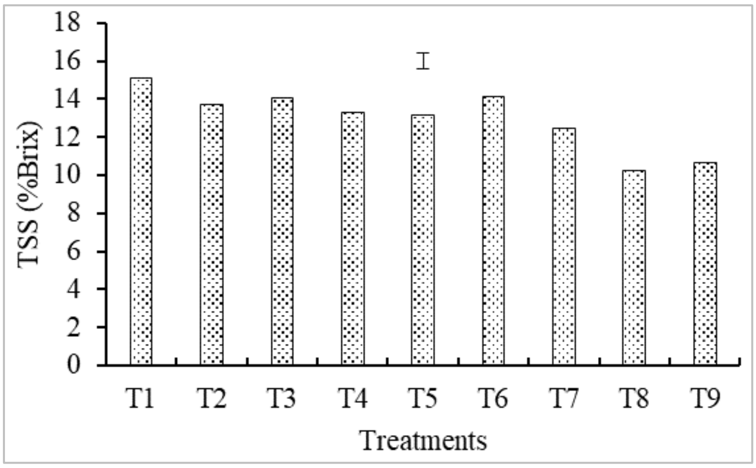

Figure 4: Effect of different levels of Gamma irradiation and Ethyl Methane Sulphonate on Total Soluble Solids

Vertical bar represents LSD at 5% level of probability; T1= GD50, T2= GD100, T3= GD150, T4= GD200, T5= ED 0.1%, T6= ED 0.3% T7= ED0.5%T8=ED0.7%, T 9= Control. GD= Doses of Gamma Radiation, ED= Doses of Ethyl methan sulphonateTSS= Total Soluble Solids

4. Conclusion

From the findings of this study, it is concluded that a significant variation existed due to the effect of different doses of treatments on the growth and yield of onion. In case of gamma irradiation dosage, T4(GD200) performed better followed by T2(GD100) is a effective dosage considering growth and yield contributing characters (bulb diameter, neck diameter, single bulb weight, TSS of onion. In case of EMS dosage, T5 (0.1%) performed better followed by T6(0.3%) is a effective dosage considering growth and yield contributing character (plant height, total bulb yield, TSS) of onion.

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