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**Research Article**

# Radio sensitivity studies in white seeded sesame (*Sesamum indicum* L.)

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**Abstract**

Sesame is an quality edible oilseed crop with the ability to grow well under drought. Seeds of two white seeded sesame varieties *viz.,* SVPR 1 and VRI 3 were irradiated with gamma ray at the following doses *viz.*, 100, 200, 300, 400 and 500 Gy. Morphological, physiological and biochemical variations were observed in the irradiated population. All morphological parameters decreased with increased dose of gamma radiation in both varieties. In biochemical traits, photosynthetic pigments increased at lower doses in both varieties. Soluble protein and proline content recorded significant changes in intermediate and higher doses in both varieties. All quantitative traits except days to 50 % flowering recorded significant decrease in higher doses in both varieties. SVPR 1 was found to be more sensitive to gamma radiation than VRI 3. Lower doses of radiation had positive effect on the biochemical and quantitative traits in both varieties. In white seeded sesame, it was observed that doses above 300 Gy are not suitable to enhance trait values.

**Keywords**

Sesame, sensitivity, gamma radiation

## Introduction

Sesame is an ancient oilseed crop which contains high amount of quality edible oil (50-60 %). It is also enriched with high amount of nutrients like calcium, zinc, iron, potassium and vitamins (Pusadkar *et al.*, 2015). The natural antioxidants sesamin and sesamolin present in sesame have anti cancerous property and are also responsible for the long storage life of oil (Mak *et al.*, 2011). Sesame is a drought tolerant crop that provides good yield even with low inputs and is well adaptable for rainfed cultivation. Sesame seeds are highly tolerant to radiation compared to other crops (Cagirgan, 2001). In general sensitivity of seeds to radiation changes with genotypes; but in sesame the seed coat colour also affects the sensitivity to radiation. Generally black seeded sesame is more tolerant to radiation than white seeded sesame (Ashri, 2001).

Gamma radiation is predominantly used in mutational breeding because of its high penetration ability into the cell. The direct effect of gamma radiation damages the DNA through ionization. Molecules in ionic state are chemically unstable and lead to mutation effect. The indirect effect of gamma radiation leads to formation of free radicals and peroxides which are highly unstable and reactive (Spencer-Lopes *et al.*, 2018). Gamma radiation significantly affects morphological and biochemical traits of the plant. Studies have been reported that lower doses of gamma ray is effective in increasing enzyme activity (Lopes *et al.*, 2017), stress tolerance (Wang *et al.*, 2018) and germination rate (Ali *et al.*, 2018). Generally, gamma radiation negatively affects root length, shoot length, fresh weight, dry weight and pollen fertility of plants (Majeed *et al*., 2018). In some cases, positive effect have been reported on germination, seed weight, proline content, total protein content, total phenol content (Hanafy and Akladious, 2018) and chlorophyll content (Singh, 1971).

This study aimed to assess the radio sensitivity of two white seeded sesame varieties to gamma radiation and to analyse the effects of gamma irradiation on morphological, physiological and biochemical traits.

## Materials and Methods

Two white seeded sesame varieties *viz.*, SVPR 1 and VRI 3 were taken for the radio sensitivity study. 5000 seeds of the both varieties were irradiated with gamma radiation at Bhabha Atomic Research Centre (BARC), Mumbai at different doses *viz.,* 100, 200, 300, 400 and 500 Gy and raised in pots and in field at the central farm of Agricultural College and Research Institute, Madurai during *kharif* 2018. Data analysis were carried out by using AGRES software.

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Gamma irradiated seeds were raised in pots with three replications in green house. Germination percentage was recorded at daily interval from 4 DAS to determine the speed of germination. Final count on germination and hypocotyl length were recorded at 10 DAS. Survival percentage of seedlings was recorded on 30th day after sowing. Root length, shoot length, fresh weight of root and shoot and dry weight of root and shoot were recorded on the 15th day after sowing.

Gamma irradiated seeds were also raised in the field in Randomized Block Design with two replications. Recommended spacing, nutrition and irrigation were provided during the crop growth period (TNAU/Agritech, 2014). Quantitative characters *viz.*, plant height, number of primary branches, number of secondary branches, number of capsules per plant, 1000 seed weight and single plant yield were recorded at maturity on ten randomly selected plants in each replication. Days to 50 % flowering was recorded on plot basis.

Chlorophyll a, chlorophyll b and carotenoid content of Fresh leaf samples collected at 15 DAS were estimated by Acetone method (Yoshida *et al.*, 1971). OD Values were recorded by sphectrophotometer at 480, 510, 645, 652 and 663 nm. Pigments content estimated by using the following formula.

$$\textbf{Chlorophyll 'a'} = 12.7\,(\mathrm{OD.}\,663) - 2.69\,(\mathrm{OD.}\,645) \times \frac{V}{1000 \times W}$$

$$\textbf{Chlorophyll 'b'} = 22.9\,(\mathrm{OD.}\,645) - 4.63\,(\mathrm{OD.}\,663) \times \frac{V}{1000 \times W}$$

$$\textbf{Total chlorophyll} = \frac{\mathrm{OD.}\,652}{34.5} \times \frac{V}{W}$$

$$\textbf{Carotenoid} = 7.6\,(\mathrm{OD.}\,480) - 1.49\,(\mathrm{OD.}\,510) \times \frac{V}{1000 \times W}$$

where,
OD- Optimal density value ; V – Final volume of supernatant
W – Weight of the leaf sample taken in gram

Fresh leaf samples (500 mg) were collected 15 DAS and the soluble protein content was estimated by Lowry's method. Bovine serum albumin (BSA) was used as standard. Absorbance value was recorded using sphectrophotometer at the wavelength of 660 nm. The absorbance value plotted in the standard graph against the corresponding concentration (X µg) recorded. Amount of soluble protein content was estimated using the following formula (Lowry *et al.*, 1951).

$$\textbf{Amount of soluble protein} = \frac{X}{10} \times \frac{25}{500} \times 1000$$

500 mg fresh leaf sample was collected 15 DAS. Proline content was estimated by the method described by Bates *et al.* (1973). Absorbance of the coloured solution was measured sphectrophotometer at 520 nm. Proline content (µg/ g FW) in the sample was estimated using the following formula.

$$\textbf{Amount of proline} = \frac{X}{2} \times \frac{10}{500} \times 1000$$

**Results and Discussion**

Gamma radiation severely altered morphological and physiological characters in both varieties. Germination and survival percentage decreased with increasing doses of radiation. The lowest germination of 42 % and survival of 36 % was recorded in 500 Gy in VRI 3 and 30 % and 26 % in SVPR 1 (Table 1). SVPR 1 was found to be more sensitive to gamma radiation in terms of germination and survival percentage. Similar results of reduction in germination and survival in increased doses of gamma radiation have been reported by Boureima *et al.* (2009) and Rajput *et al.* (2001).

Hypocotyl length decreased with increased dose of gamma radiation. In SVPR 1 and VRI 3 hypocotyl length reduced gradually with increasing doses of gamma radiation and recorded the lowest value of 1.24 cm and 2 cm respectively at 500 Gy of gamma radiation. Hypocotyl length was found to be positively correlated with survival percentage of seedlings. These results were similar to the results observed by Murty (2001).

Pollen fertility was observed to be 90 % and 92.5 % in unirradiated control of SVPR 1 and VRI 3. As the gamma radiation dose increased, pollen fertility reduced gradually and was observed to be 40.05 % and 41.5 % in SVPR 1 and VRI 3 at 500 Gy of gamma radiation (Table 1). Reduction in pollen fertility due to gamma radiation was reported by previous workers by Kumar and Yadav (2010), Bashir *et al.* (2013) and Ariraman *et al.* (2018).

Root length and shoot length of seedling decreased with increase in dose of radiation (Table 2). Similar results were reported by Pathirana and Subasinghe (1993). According to Oney-Birol and Balkan (2019) decrease in growth rate at high dose of gamma irradiation was due to cell cycle arrest during $G_2$/M phase during somatic cell division and variety of damages in entire genome. Auxin destruction and physiological and biochemical disturbances could lead to inhibition of germination and plant development (Shah *et al.*, 2008). Maximum reduction was observed in 500 Gy of gamma radiation in both varieties. Reduction of root length and shoot length was higher in SVPR 1 than VRI 3 (Table 2).

Fresh weight and dry weight of shoot and root of seedlings were recorded 15 DAS. Fresh weight and dry weight of root and shoot decreased with increasing dose of radiation. Pathirana and Subasinghe (1993) reported similar results. They observed that increase in gamma ray doses from 250 to 1750 Gy resultedin decrease in fresh and dry weight of root and shoot. Dry weight indicates the bio mass of the seedlings. In control, fresh weight and dry weight of SVPR 1 was higher than that of VRI 3. But with increased radiation, the reduction level of fresh weight and dry weight of the seedling was higher in SVPR 1, in most of the cases.

This suggests that SVPR 1 is more sensitive to gamma radiation compared to VRI 3. In both varieties the weight of seedlings decreased with increased dose of radiation, which is in agreement with the results of Pathirana and Subasinghe (1993). Reduction in fresh weight and dry weight of shoot may be attributed to reduction in shoot moisture content due to radiation stress (Majeed *et al*., 2018).

Photosynthetic pigments and carotenoid content increased in lower doses of radiation and then started to decline when the doses increases. (Table 3). In SVPR 1, chlorophyll a and chlorophyll b content recorded the maximum value of 0.83 and 0.54 mg/g FW respectively at 200 Gy. In VRI 3, the maximum value of chlorophyll a and chlorophyll b were recorded at 300 Gy which was 0.85 and 0.57 mg/g FW, respectively. Chlorophyll a and b content increased upto 200 Gy in SVPR 1 and 300 Gy in VRI 3; thereafter it gradually decreased in both varieties. Similar results were reported by Singh (1971). He observed that chlorophyll content increased upto 200 Gy then it gradually decreased. Marcu *et al.* (2013) reported that chlorophyll b decreased with increasing gamma ray doses. In addition, it was observed that chlorophyll a was relatively higher than chlorophyll b in irradiated and control seedlings. Kiong *et al.* (2008) and Marcu *et al.* (2013) attributed this to greater destruction of chlorophyll b than chlorophyll a due to disturbance in its biosynthesis.

SVPR 1 and VRI 3 recorded maximum carotenoid content of 0.34 and 0.36 mg/g FW at 200 Gy (Table 3). In both varieties the carotenoid content increased upto 200 Gy; thereafter it gradually decreased. Marcu *et al.* (2013) reported that carotenoid content decreased with increased dose of gamma radiation. Soluble leaf protein content in control sample of SVPR 1 and VRI 3 was 15 and 12 mg/g FW. The maximum value of soluble protein content observed in SVPR 1 was 20.5 mg/g FW at 300 Gy (Table 3). In VRI 3, the maximum value of 14 mg/g FW was recorded at 200 Gy. Soluble protein level increased upto 300 Gy in SVPR 1 and 200 Gy in VRI 3; then it gradually decreased in both the varieties. Afify *et al.*, 2011 reported increase in gamma radiation decreased total soluble protein content in sesame. Increase in soluble protein at lower doses of gamma radiation is one of the protective mechanisms of the plant against gamma irradiation (Al-Rumaih and Al-Rumaih, 2008). Reduction in total protein with increasing gamma ray dosage may be due to higher metabolic and hydrolysing enzyme activities (Maity *et al.*, 2004).

Proline acts as osmoregulator via tolerance and protection against various stresses (Kuznetsov *et al.*, 2003). The results obtained indicated that proline content was increased by various doses of gamma rays. Maximum proline content of 0.58 µg/g FW and 0.6 µg/g FW was recorded in SVPR 1 and VRI 3 at 300 and 200 Gy respectively (Table 3). These results are in accordance with the experts of Borzouei *et al.* (2010) who also observed increase in proline upto 300 Gy of gamma radiation. Hanafy and Akladious (2018) observed an increase in proline content upto 200 Gy of gamma radiation.

Plant height at maturity decreased with increased dose of radiation (Table 4). At 500 Gy plant height recorded the lowest value of 67 cm in SVPR 1 and 65 cm in VRI 3. Number of primary branches recorded positive significant value in 100, 200 and 300 Gy in SVPR 1 and 100 and 200 Gy in VRI 3. For secondary branches positive significant value was observed in 100 and 300 Gy in SVPR 1 and 100 and 200 Gy in VRI 3. Hussein and Hamideldin (2016) reported that plant height increased upto 90 Gy of gamma radiation in sesame. B. Singh and Datta (2010) observed improvement in plant growth, yield, flag leaf area and photosynthesis at lower doses of gamma ray in wheat. Days to 50 % flowering was observed earlier than control in 300 and 400 Gy in both varieties. Boureima *et al.* (2009) reported days to first flowering increased with increased dose of radiation.

Number of capsules per plant decreased with increased dose of radiation in both varieties. The least number of capsules were recorded at 500 Gy in SVPR 1 (57.66) and VRI 3 (55.33). 1000 seed weight was not much affected by gamma radiation but significant increase in 1000 weight was recorded at 400 Gy in SVPR 1. Single plant yield decreased with increased dose of radiation in both varieties. Hussein and Hamideldin (2016) reported that number of capsules and single plant yield increased upto 60 Gy of gamma radiation.

From this study it is concluded that low doses of gamma radiation have positive effect on the biochemical constituents and quantitative traits of sesame concomitant with induction of non-enzymatic antioxidant compounds. Higher doses of gamma radiation had an inhibitory effect due to production of free radicals which negatively affected all the traits according to irradiation level. Biochemical traits enhanced effectively upto 200 Gy in SVPR 1 and 300 Gy in VRI 3. Between the two varieties studied, SVPR 1 was found to be more sensitive to radiation than VRI 3. Also SVPR 1 responded well to all doses of gamma radiation in terms of altered morphological, physiological and biochemical traits.

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**Table 1. Effect of gamma radiation on germination, hypocotyl length, survival percentage and pollen fertility**

| Gamma ray doses (Gy) | Germination (%) | | HL 10 DAS (cm) | | Survival percentage (%) | | Pollen fertility (%) | |
|---|---|---|---|---|---|---|---|---|
| | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 |
| **Control** | 85 | 90 | 6.8 | 7.82 | 85 | 90 | 90 | 92.5 |
| **100** | 75 $^{*}$ | 79 $^{**}$ | 5.2 | 5.9 | 67 $^{**}$ | 72 $^{**}$ | 75.80 $^{**}$ | 72.5 $^{**}$ |
| **200** | 55 $^{**}$ | 72 $^{**}$ | 3.6 | 6.5 | 52 $^{**}$ | 67 $^{**}$ | 72.05 $^{**}$ | 65.80 $^{**}$ |
| **300** | 43 $^{**}$ | 65 $^{**}$ | 3.4 | 4.3 | 39 $^{**}$ | 60 $^{**}$ | 65.45 $^{**}$ | 54.25 $^{**}$ |
| **400** | 38 $^{**}$ | 55 $^{**}$ | 2.91 | 3.4 | 35 $^{**}$ | 51 $^{**}$ | 46.74 $^{**}$ | 47.02 $^{**}$ |
| **500** | 30 $^{**}$ | 42 $^{**}$ | 1.24 | 2.0 | 26 $^{**}$ | 36 $^{**}$ | 40.05 $^{**}$ | 41.5 $^{**}$ |
| **Mean** | 54.33 | 67.17 | 3.86 | 4.99 | 50.67 | 62.67 | 65.02 | 62.26 |
| **CD at 1 %** | 11.94 | 9.46 | 1.06 | 1.18 | 8.78 | 10.17 | 10.28 | 10.32 |
| **CD at 5 %** | 8.63 | 6.84 | 0.76 | 0.85 | 4.12 | 7.35 | 7.43 | 7.46 |

**$^{*}$, $^{**}$ indicates CD at 5 % and 1 % level of significance. ns- non significant**

**Table 2. Effect of gamma radiation on morphological traits of sesame**

| Gamma ray doses (Gy) | Shoot length (cm) | | Root length (cm) | | Shoot FW (mg) | | Root FW (mg) | | Shoot DW (mg) | | Root DW (mg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 |
| **Control** | 8.8 | 11.5 | 10.4 | 10 | 282.1 | 242.3 | 49.9 | 48.1 | 33.3 | 30 | 5.7 | 4.4 |
| **100** | 7.5 $^{*}$ | 7.4 $^{**}$ | 7.7 $^{*}$ | 8.1 $^{*}$ | 212.5 $^{*}$ | 210.2 $^{*}$ | 42.5 $^{*}$ | 43.2 $^{*}$ | 30.2 $^{*}$ | 28.7 $^{ns}$ | 4.2 $^{ns}$ | 3.9 $^{ns}$ |
| **200** | 7.1 $^{*}$ | 5.7 $^{**}$ | 6.5 $^{**}$ | 6.42 $^{**}$ | 152.7 $^{**}$ | 186.7 $^{**}$ | 35.2 $^{**}$ | 38.2 $^{**}$ | 27.3 $^{*}$ | 24.7 $^{**}$ | 3.5 $^{*}$ | 3.5 $^{*}$ |
| **300** | 5.1 $^{**}$ | 5.3 $^{**}$ | 5.4 $^{**}$ | 5.6 $^{**}$ | 140.9 $^{**}$ | 174.3 $^{**}$ | 32.1 $^{**}$ | 33.3 $^{**}$ | 21.73 $^{**}$ | 19.8 $^{**}$ | 2.45 $^{**}$ | 2.7 $^{*}$ |
| **400** | 3.0 $^{**}$ | 4.3 $^{**}$ | 3.1 $^{**}$ | 4.5 $^{**}$ | 78.7 $^{**}$ | 169.8 $^{**}$ | 10.9 $^{**}$ | 26.5 $^{**}$ | 8.35 $^{**}$ | 14.1 $^{**}$ | 1.6 $^{**}$ | 1.5 $^{**}$ |
| **500** | 1.7 $^{**}$ | 2.6 $^{**}$ | 2.3 $^{**}$ | 3.7 $^{**}$ | 69.2 $^{**}$ | 74.4 $^{**}$ | 8.3 $^{**}$ | 12.53 $^{**}$ | 6.1 $^{**}$ | 8.4 $^{**}$ | 0.37 $^{**}$ | 0.9 $^{**}$ |
| **Mean** | 5.37 | 6.13 | 5.90 | 6.39 | 156.02 | 176.28 | 29.82 | 33.64 | 21.16 | 20.95 | 2.97 | 2.82 |
| **CD at 1 %** | 1.46 | 1.69 | 1.65 | 1.29 | 44.51 | 31.12 | 9.25 | 7.03 | 6.30 | 4.68 | 1.05 | 0.76 |
| **CD at 5 %** | 1.05 | 1.22 | 1.19 | 0.93 | 32.17 | 22.50 | 6.69 | 5.08 | 4.56 | 3.38 | 0.76 | 0.55 |

**$^{*}$ and $^{**}$ indicates CD at 5 % and 1 % level of significance. ns- non significant**

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**Table 3. Effect of gamma radiation on biochemical traits of sesame**

| Gamma ray doses (Gy) | Chlorophyll a (mg/g FW) | | Chlorophyll b (mg/g FW) | | Total Chlorophyll (mg/g FW) | | Carotenoid (mg/g FW) | | Soluble protein (mg/g FW) | | Proline (µg/g FW) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 | SVPR 1 | VRI 3 |
| **Control** | 0.55 | 0.63 | 0.37 | 0.48 | 0.92 | 1.11 | 0.24 | 0.31 | 14.98 | 11.95 | 0.44 | 0.50 |
| **100** | 0.71 $^{**}$ | 0.70 $^{**}$ | 0.44$^{*}$ | 0.5 $^{ns}$ | 1.15$^{**}$ | 1.2$^{*}$ | 0.29 $^{*}$ | 0.34$^{**}$ | 16.5$^{**}$ | 12.93$^{*}$ | 0.38$^{**}$ | 0.55$^{**}$ |
| **200** | 0.83 $^{**}$ | 0.73 $^{**}$ | 0.54$^{**}$ | 0.54$^{**}$ | 1.37$^{**}$ | 1.27$^{**}$ | 0.34$^{**}$ | 0.36$^{**}$ | 18.6$^{**}$ | 14 $^{**}$ | 0.46 | 0.60$^{**}$ |
| **300** | 0.68 $^{**}$ | 0.85 $^{**}$ | 0.44$^{**}$ | 0.57$^{*}$ | 1.12$^{**}$ | 1.42$^{**}$ | 0.27 $^{**}$ | 0.3 $^{ns}$ | 20.5$^{**}$ | 13.08 $^{**}$ | 0.58$^{**}$ | 0.44$^{**}$ |
| **400** | 0.63 $^{**}$ | 0.79 $^{**}$ | 0.41$^{**}$ | 0.51$^{*}$ | 1.04$^{**}$ | 1.3$^{**}$ | 0.29 $^{**}$ | 0.32 $^{ns}$ | 15.54 | 11.86 | 0.44$^{ns}$ | 0.46$^{**}$ |
| **500** | 0.50 $^{*}$ | 0.50 $^{**}$ | 0.32$^{**}$ | 0.36$^{**}$ | 0.82$^{*}$ | 0.86$^{**}$ | 0.20 $^{*}$ | 0.22$^{**}$ | 12.6$^{**}$ | 8.5 $^{**}$ | 0.42 | 0.46$^{*}$ |
| **Mean** | 0.65 | 0.70 | 0.42 | 0.49 | 1.07 | 1.19 | 0.27 | 0.31 | 16.45$^{*}$ | 12.05 | 0.45 | 0.50 |
| **CD at 1 %** | 0.07 | 0.07 | 0.04 | 0.04 | 0.11 | 0.11 | 0.03 | 0.03 | 1.54 | 1.06 | 0.04 | 0.03 |
| **CD at 5 %** | 0.05 | 0.05 | 0.03 | 0.03 | 0.08 | 0.08 | 0.02 | 0.02 | 1.11 | 0.77 | 0.03 | 0.02 |

*** ** indicates CD at 5 % and 1 % level of significance. ns- non significant**

**Table 4. Effect of gamma radiation on quantitative traits of sesame**

| Gamma ray doses (Gy) | Plant height at maturity | | No. of Primary Branches | | No. of secondary branches | | Days to 50 % flowering | | No. of capsules per plant | | 1000 seed weight (g) | | Single plant yield (g) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **SVPR 1** | **VRI 3** | **SVPR 1** | **VRI 3** | **SVPR 1** | **VRI 3** | **SVPR 1** | **VRI 3** | **SVPR 1** | **VRI 3** | **SVPR 1** | **VRI 3** | **SVPR 1** | **VRI 3** |
| **Control** | 120.5 | 115.58 | 2.75 | 3.06 | 3.1 | 3.25 | 35 | 36.5 | 87.5 | 80.25 | 3.56 | 3.25 | 9.37 | 9.2 |
| **100** | 92.6 $^{**}$ | 89.5 $^{**}$ | 3.46 $^{**}$ | 3.25$^{**}$ | 3.85$^{**}$ | 3.45$^{*}$ | 38.5$^{**}$ | 39$^{**}$ | 88 $^{ns}$ | 82 $^{ns}$ | 3.65$^{*}$ | 3.13 $^{**}$ | 8.10$^{**}$ | 8.2$^{**}$ |
| **200** | 83.5 $^{**}$ | 80.2 $^{**}$ | 3.25 $^{**}$ | 3.15$^{*}$ | 3.16$^{ns}$ | 3.6$^{**}$ | 42.5$^{**}$ | 40.5$^{**}$ | 84 $^{ns}$ | 75.33 $^{*}$ | 3.45$^{**}$ | 3.05 $^{**}$ | 7.9$^{**}$ | 7.89$^{**}$ |
| **300** | 77.25 $^{**}$ | 75.6 $^{**}$ | 3.5 $^{**}$ | 2.85$^{**}$ | 3.33$^{**}$ | 2.85$^{**}$ | 33$^{*}$ | 31$^{**}$ | 79 $^{**}$ | 67.33 $^{**}$ | 3.63$^{**}$ | 2.87 $^{**}$ | 7.35$^{**}$ | 7.75$^{**}$ |
| **400** | 74.09 $^{**}$ | 69.5 $^{**}$ | 2.33 $^{**}$ | 2.75$^{**}$ | 2.53$^{**}$ | 3.1$^{*}$ | 31.5$^{**}$ | 30.5$^{**}$ | 63 $^{**}$ | 63.66 $^{**}$ | 3.68$^{**}$ | 2.75 $^{**}$ | 7.25$^{**}$ | 6.9$^{**}$ |
| **500** | 67 $^{**}$ | 65 $^{**}$ | 2.25 $^{**}$ | 2.5$^{**}$ | 2.7$^{**}$ | 2.4$^{**}$ | 41.83$^{**}$ | 39.5$^{**}$ | 57.66 $^{**}$ | 55.33 $^{**}$ | 3.25$^{**}$ | 2.95 $^{**}$ | 6.33$^{**}$ | 6.5$^{**}$ |
| **Mean** | 85.82 | 82.56 | 2.92 | 2.93 | 3.11 | 3.11 | 37.06 | 36.17 | 76.53 | 70.65 | 3.54 | 3.00 | 7.72 | 7.74 |
| **CD at 1 %** | 10.53 | 10.05 | 0.31 | 0.16 | 0.26 | 0.24 | 2.57 | 2.43 | 7.19 | 5.73 | 0.10 | 0.11 | 0.57 | 0.53 |
| **CD at 5 %** | 7.61 | 7.26 | 0.22 | 0.11 | 0.19 | 0.17 | 1.86 | 1.75 | 5.20 | 4.14 | 0.07 | 0.08 | 0.41 | 0.39 |

***, ** indicates CD at 5 % and 1 % level of significance. ns- non significant**