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**(Research Article)**

# Effect of Heat Treatment on Germination, Seedling Growth and Some Biochemical Parameters of Dry Seeds of Black Gram

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**ABSTRACT**

*Seeds have been extensively used for studying the effect of temperature on physiological process. Dry seeds are frequently able to withstand a broad range of temperature but after the germination has been set in motion by the inhibition of water most seeds appear to tolerate a much narrower range of temperatures. Many of the early studies led to the concept of minimal, maximal and optimal temperatures for seed germination and biochemical changes. At first, the effect of heat treatment of dry seeds of black gram at 50$^{O}$C for 10, 20 and 30 min. duration on seed germination was conducted. Then, next experiment was the 3 day old seedlings also were given the same treatment with control to find out the effect on further growth and biochemical changes. Analysis showed, the duration of 30 min. (50$^{O}$C) was found to be significantly effective in reducing the seed germination, seedling growth and vigour index. Biochemical study on protein and proline content found to be markedly high in 30 min. treated than other two treatments, whereas chlorophyll content was high in the samples of 10 and 20 min. treated seedlings. Our results indicate that, heat treatment at 50$^{O}$C to black gram seeds could tolerate and germinate into normal seedlings for short duration( 10 and 20 min.) and in biochemical analysis, protein and proline content was high in 30 min.(50$^{O}$C) duration and chlorophyll was very low.*

**Key Words:** Black gram, Heat treatment, Biochemical analysis, Protein , Seed germination, Vigour Index

## INTRODUCTION

Seeds of many different species native to temperature regions will fail to germinate until after they have been exposed in a moist condition to temperatures between 0$^{O}$ and about 6$^{O}$C for a few weeks to several months. During this period of exposure to low temperatures, changes in metabolism take place, after the completion of which the seeds will germinate promptly if placed in a favorable situation[5]. The importance of temperature is prolonging or breaking of dormancy. Freshly harvested seeds which have high temperature dormancy exhibit higher percentage germination at low temperature than at high temperature [2]. For example, single alternation from 15$^{O}$C to 25$^{O}$C in connection with light treatment of pepper grass seeds may significantly increase germination[6].

Alternating temperatures may have been required for the destruction of germination inhibitors and / or for the synthesis or activation of germination stimulators. High temperatures are usually responsible for the destruction of inhibitors in plants [8], although in some seeds their accumulation is retarded by low temperatures. The synthesis and activity of some plant enzymes, e.g., the mobilization of carbohydrates and proteins in the seed of *Heracleum sphondylium* L.[12], and starch hydrolysis and phosphorylase activity in potato tubers[1], are usually greater at low temperatures, although some investigators have suggested that high temperatures may be necessary for adequate release of energy for germination and growth[3, 13, 14].

In the present investigation, an attempt was made to study the effect of treatment of Black Gram dry seeds at $50^{O}$C for 10, 20 and 30 min. duration on seed germination, biochemical changes and in addition to the 3 day old seedlings also was given the same treatment to find out the temperature effect on further growth.

## MATERIALS AND METHOD

Pure line black gram (*Vigna mungo* T-9) seeds obtained from Agricultural Station at Tiruchirappalli were used as the experimental material. Healthy seeds in batches were treated with 0.1% mercuric chloride for 1 min. for surface sterilization and then washed with distilled water. Surface water was very quickly absorbed by a blotting paper and termed as dry seeds. The seeds after washing with distilled water were subjected to heat shock treatment by placing in separate beakers containing hot water maintained in thermostat at $50^{O}$C for 10 min.20 min. and 30 min. respectively. Another seed lot was kept in distilled water at room temperature as control (30 min.). All heat treated and control seed lots were then spread over moist filter paper in separate petridishes and allowed to germinate in humid atmosphere at room temperature for 5 days. Simultaneously, 3 day old seedlings were given same treatment ($50^{O}$C) for 3 durations (10, 20 and 30 min.).

## MORPHOLOGICAL EVALUATION

The following morphological parameters were investigated:

### Seed Germination

Seed germination study was carried out on the 5$^{th}$ day after sowing. From all the varieties, the number of seeds germinated were counted and recorded. Germination percentage was worked out by the following method

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated X 100}}{\text{Total number of seeds sown}}$$

### Length of the Root

The root length was taken on the 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ day after sowing by selecting 5 seedlings at random from all the treatments. The average value was recorded in centimeters.

### Length of the Shoot

Shoot length was recorded on the 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ day after sowing for each treatment. The shoot length was measured for randomly selected five seedlings and the average value was recorded.

### Vigour Index

Observations for the germination, root length and vigour index were made on the 5$^{th}$ day after sowing. Vigour index was calculated using the following formula.
VI= (root length+ shoot length) x percentage of germination.

### Cotyledons weight

Fresh weight of cotyledons was taken from 5 randomly selected seedlings on the 5$^{th}$ day after germination from all the treatments. The average weight of cotyledons per seedling was recorded in milligrams.

## BIOCHEMICAL ANALYSIS

The seedlings of different treatments were taken for the following biochemical analysis:

### Estimation of total soluble protein (Lowry *et al*., 1951)

Protein was extracted using seedling of known weight by homogenizing it with 5 ml of ice cold phosphate buffer. The homogenate was filtered through the filter paper. To the filtrate equal volumes of 10% TCA was added and centrifuged for 10 minutes. The sediment which constituted the protein was dissolved in 1 ml on 0.1N NaOH solution. To this protein extract, 4 ml of alkaline solution was added and mixed well and allowed to stand at room temperature for 10 min. Then 0.5 ml of 1:2 folin ciocalteau solutions was added and after 10 min. the optical density of the solution was taken at 660 nm using ERMA colorimeter. Blank was also prepared. Protein content was calculated by referring to a standard curve and the results are expressed as mg /g of fresh tissue.

**Proline Determination**

Proline was extracted using seedling of known weight by homogenizing it with 10 ml of sulfosalicylic acid. The homogenate was filtered through the Whatman No.2 filter paper. The extraction was repeated and the filtrate was pooled. From the filtrate, 2 ml was taken into a test tube; 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added and incubated for 1 hr at $100^{O}$C in water bath. The test tubes were transferred to an ice bath to terminate the reaction. 4 ml of toluene was added and mixed vigorously using a test tube stirrer for 15 to 20 seconds. The chromophore containing toluene was aspirated from the aqueous phase. It was allowed to thaw at room temperature and the absorbance was measured at 520nm. A reagent blank was maintained. The proline concentration was determined from a standard curve prepared with authentic proline and calculated its amount on fresh weight basis.

**Chlorophyll Content**

The chlorophyll content was estimated by a procedure outlined by Arnon (1949).

50 mg of fresh leaves were homogenized with 3 ml of 80% acetone. The homogenate was centrifuged at 4000 rpm for 5 min. and the final volume was made up to 10 ml with 80% acetone. OD of the extract was read at 663 nm and 645 nm in the Spectronic 20. Chlorophyll contents of the samples were calculated using the following formula.

$$\text{Total chlorophyll in mg/g} = 20.2(A645) + 8.02(A663) \times \frac{V}{1000xW}$$

Where,

A= absorbance at specific wave lengths

V= final volume of chlorophyll extract in 80% acetone

W= fresh weight of tissue extracted.

**RESULTS AND DISCUSSION**

**Seed Germination**

The seeds subjected to different heat stress showed variation in germination percentage in different durations of treatment. In general, heat treatment promoted seed germination in black gram. The highest seed germination of 93 percentages was registered for the seed that were treated at $50^{O}$C for 20 min. It was followed by the seeds that were treated for 10 min (88%). It was 80% in 30 min. duration. The least germination percentage was recorded in the seeds of control which were not given in any heat stress (Fig .1).

As for as seed germination is concerned in the present study, treatment of dry seeds at $50^{O}$C to different durations (10, 20 and 30 min.) had a positive effect on the germinability of the black gram seeds. It was much pronounced in the seeds that were given heat stress for about 20 min. Seeds germination studies using different temperature treatment in earlier studies showed promotion in seed germination. Dry seeds were reported to be resistant to broad range of temperature in number of monocotyledons seed[5]. It was reported in cantaloupe that it required higher temperature for seed germination and tolerated temperatures between 45 and $50^{O}$C[4].

Similar to seed treatments, 3 day old seedlings were also given heat stress at $50^{O}$C for three durations (10, 20, and 30 min.). Following heat treatment, seedlings that were treated for 20 and 30 min. dried and further growth was inhibited completely. However, the seedlings that received heat treatment ($50^{O}$C) for 10 min. showed damage of seedlings to some extent and few seedlings survived up to two days. Then they also started drying on the subsequent days (Fig.2).

**Length of Root and Shoot**

The length of primary root was measured from $2^{nd}$ day after sowing the seeds. The shoot length was measured from $3^{rd}$ day onwards up to $5^{th}$ day. The root length on the initial day was 2.3cm in the seeds that were treated for 10 min. at $50^{O}$C. It was the least (1cm) in 30 min. treated. An increase in the growth of root and shoot was observed from the day of analysis up to $5^{th}$ day. When the root length of the treated was compared, promotion in the growth was observed in 10 & 20 min. duration. However, 30 min. duration not only inhibited seed germination but also reduced the growth and the root length. The seeds that were not given heat treatment (control) showed less growth and the root length was shorter than the heat treated seeds (10 & 20 min.). Approximately the same response was recorded regarding the shoot growth of the black gram seedlings in control of treatment (Fig. 3, 4 and 5).

The growth was much pronounced in seeds that were given heat stress for 20 min. for $50^{O}$C, followed by 10 min. duration. The shoot growth was highly reduced in 30 min. duration of heat stress at $50^{O}$C. The shoot growth in control was only moderate. The rate of elongation of pea roots and maize coleoptiles was reported to

be linear with the increase of temperature up to $30^{O}$C and above this temperature, the rate of growth decreased sharply in those plants[10]**.** However in our studies, up to $50^{O}$C the black gram seeds were able to with stand and could grow well after treating them for 20 min. duration. Therefore, it could be stated from the present investigation that heat stress given to dry seeds at $50^{O}$C durations up to 20 min. had much pronounced effect on the growth characteristic of black grams.

**Vigour Index**

The seeds subjected to different heat stress were calculated for their vigour index. The highest vigour index value of 1448 was registered for the seeds that were treated in $50^{O}$C for 20 min. The seeds that were treated 10 min. showed the value to be 1199. In control it was 552. (Table.1) The least vigour index value of 301 was noted in the seeds in $50^{O}$C for 30 min. 10 & 20 min. treatments increased the vigour index. Increase in vigour index might be attributed to their better growth performance with high seed germination percentage. Remarkable reduction in vigour index in control could be due to its reduction in germination percentage and its growth. Whereas, the growth reduction at $50^{O}$C in 30min. duration could be considered as an inhibitory response of seed to high stress with long duration of exposure. Therefore it may be concluded that the seeds of black gram could tolerate temperature up to $50^{O}$C for the duration of 20 min.

**Cotyledons weight**

The seedlings were taken for analysis on the $5^{th}$ day after sowing. As the seedlings retained most part of the cotyledons, they were taken for analysis to find out the difference in fresh weight among various treated seedlings. (Table.1). The cotyledon weight was found to be 60 mg per seedling in those that were treated for 30 min. duration at $50^{O}$C. This value was the highest among the various treatments. In control, the cotyledon weight was 36 mg. In the other two treatments (10 & 20 min. durations) the cotyledon weight was 36 mg. In the other two treatments (10 & 20 min. duration) the cotyledon weight was only 28 mg. It is very obvious from these results that the cotyledon reserves was used maximum by those seedlings that were grown from 10 and 20 min. duration treatments. It could be observed from the present studies on seedling growth of black gram (Fig.3). The seedling growth in the heat stress seeds of 10 & 20 min. duration was much pronounced. The cotyledons reserves could have remarkably contributed to the growth of those seedlings when compared to the seedlings grown from 30 min. duration. The seedling of the latter showed inhibitory response with regard to the growth and their cotyledons remain fresh and large even up to $5^{th}$ day after sowing. That might be the reason for high fresh weight of their cotyledons (30 min.duration). In the control, there was only a moderate growth in seedling and the fresh weight of cotyledons was determined to be 36 mg seedling. It was less than the weight of cotyledons of seedlings grown from 30 min. duration treatment and higher than the other two heat treatments (10 & 20 min.).

**Protein**

The protein content was estimated from the entire seedling of all the treatments. It was the minimum in the seedlings of 10 min. treatment (1389µg/g). The protein content was slightly higher in the seedlings of 20 min. duration (1667µg/g). The highest amount of protein was estimated from the seedling that were grown from seeds received heat treatment ($50^{O}$C) for 30 min. (4063µg/g). In control also, it was considerably high (3500µg/g). The stunted growth of seedlings in 30 min. duration could be attributed to heat stress for long duration that might have led to poor utilization of seed reserves which resulted in the great accumulation of protein. The reduction in the level of protein in the seedlings of 10 & 20 min. duration could be accounted for by their conversion into some other organic compounds such as amino acids or growth promoting substance in those seedlings. The growth response shown by the seedlings of 10 & 20 min. duration could be taken as evidence from the present study. **Stotzky and Cox(1962)** Suggested that, high temperature may be necessary for adequate release of energy for germination and growth[13] (Fig.6).

**Proline**

It is an aminoacid which accumulates in large quantity due to any kind of stress. Temperature stress given to black gram seeds resulted in the growth of seedlings in much pronounced way when compared to the control. It was also very interesting to note that the seedlings growth from stress induced (different duration) seeds responded differently. In all the treated, the proline content was found many fold greater than the control. It was the highest in the seedlings that were grown from seeds treated for 30 min. duration at $50^{O}$C (19.9µg/g). Proline content of the seedlings showed a decline with decrease in duration of seed treatment. The seeds that received heat stress (10 and 20 min.) tolerated and produced seedlings in a normal condition. Those seedlings accumulated relatively high amount of proline to overcome stress. Accumulation of proline in plants during

various environmental stresses is a well known fact and is used as an index of stress resistance as reported by several stress physiologists[11].

Dry seeds of black gram could withstand temperature at $50^{O}C$ for up to 20 min. duration. In spite of the stress condition, those seeds were able to germinate and produce normal seedlings. However, the large quantity of proline estimated from these seedlings indicates the sign of stress produced in the seedlings. To overcome stress condition, the seeds subjected to heat treatment synthesized more proline as compared to the control in the present investigation. Studies with suspension cultured plant cells indicate that adaptation to temperature stress is closely associated with proline accumulation[9]. Proline accumulation in plant tissues can be considered as a soluble nitrogen sink. Reports are available in favour of proline synthesis due to temperature stress[7]. (Fig.7).

**Total Chlorophyll**

The total chlorophyll content of leaves from seedlings of 10 min. duration was found to be high among the treatment. It was followed by 20 min. duration. In control, the total chlorophyll content was less than the above mentioned heat treated samples (10 & 20 min.). The total chlorophyll content was very low in 30 min. Heat treatment for short durations (10 & 20 min.) showed enhancement in the growth of the seedling and the leaves produced by those seedlings that were normal and greenish in appearance as compared to the seedlings that were grown from seeds treated with 30 min. duration. Similar to other responses, the chlorophyll content also was only moderate in the leaves of control samples (Fig.8).

**CONCLUSION**

Biochemical study on protein revealed that in 10 and 20 min. treated the protein content was low in the seedlings. The growth performance was normal in those seedlings. Therefore the protein content might have been converted into other biochemical growth factors. Whereas, in 30 min. treated, the growth was severely affected and large amount of cotyledon was retained in those seedlings. Therefore, the protein content of the seedlings was found to be markedly high in 30 min. treated.

Similarly, the total chlorophyll content also was markedly high in the samples of 10 and 20 min. treated. However, the amount of proline increased sharply with the increase in the duration of time. It is started that proline is an aminoacid which accumulates to a phenomenal level in plants subjected to any stress condition. It is concluded that heat treatment at $50^{O}C$ to black gram seeds could tolerate and germinate into normal seedlings when they were treated for duration of up to 20 min. beyond that period, the seeds of black gram did not show tolerance and it resulted in the death of some seeds and few of them developed into abnormal seedlings.

**Table-1: Germination percentage, Vigour index and Cotyledon weight of black gram seedling obtained from heat treated seeds at $50^{O}C$ for different durations**

| Treatments | Germination (%) | Vigour index | Cotyledon Weight (mg) |
|---|---|---|---|
| Control | 68 | 552 | 36 |
| $50^{O}C$(10min) | 88 | 1199 | 28 |
| $50^{O}C$(20min) | 93 | 1448 | 28 |
| $50^{O}C$(30min) | 80 | 30 | 60 |

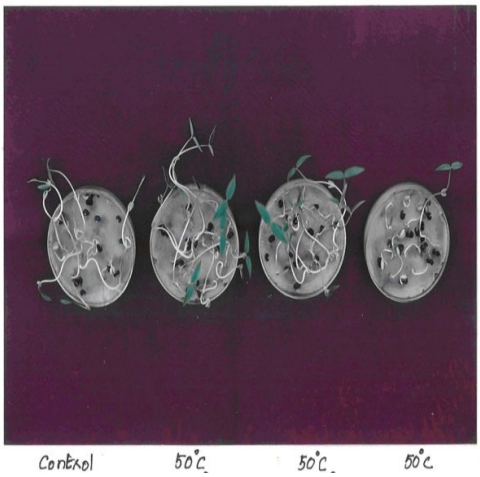


**Figure-1**


**Figure-2**

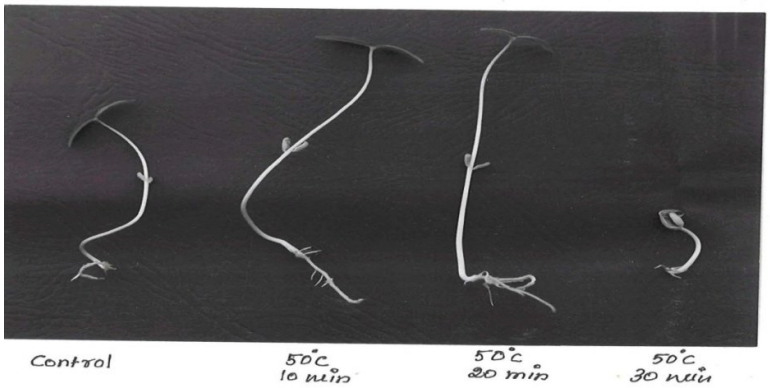


**Figure-3**

**Fig-1 : Effect of heat treatment on Germination and growth of Black gram seeds.**
**Fig-2: Effect of heat treatment of 3 day old black gram seedlings.**
**Fig-3: Effect of heat treatment on the root and shoot growth in seedlings(5 day old) of black gram seeds.**

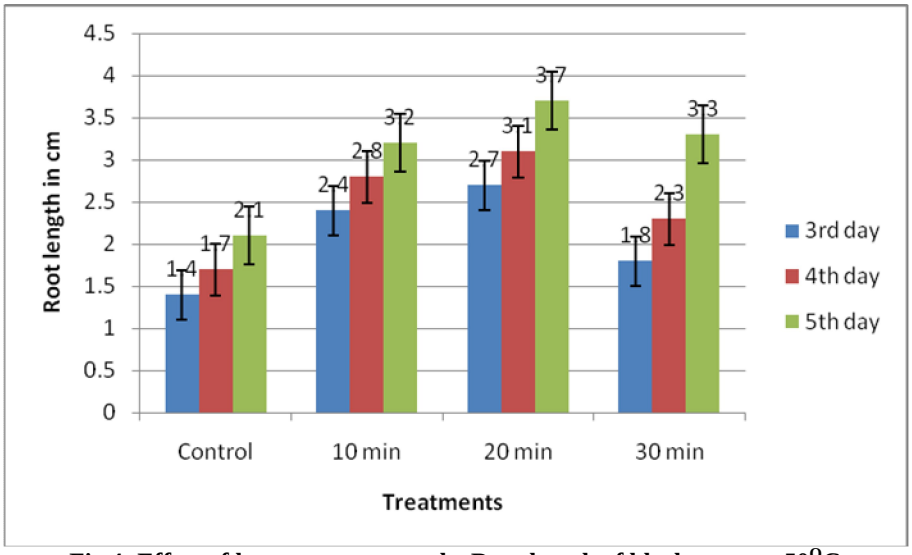


**Fig 4: Effect of heat treatment on the Root length of black gram at $50^{O}C$ for different durations**

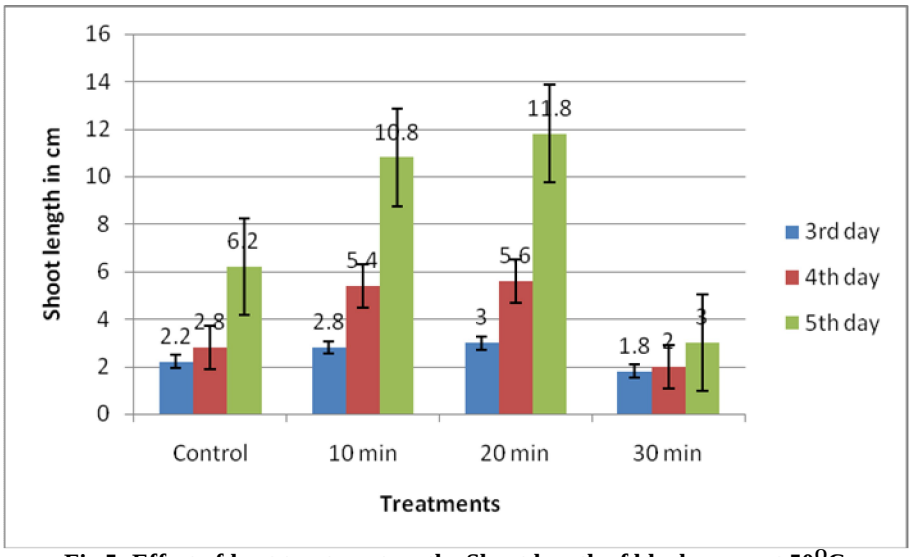


**Fig 5: Effect of heat treatment on the Shoot length of black gram at 50$^{O}$C for different durations**

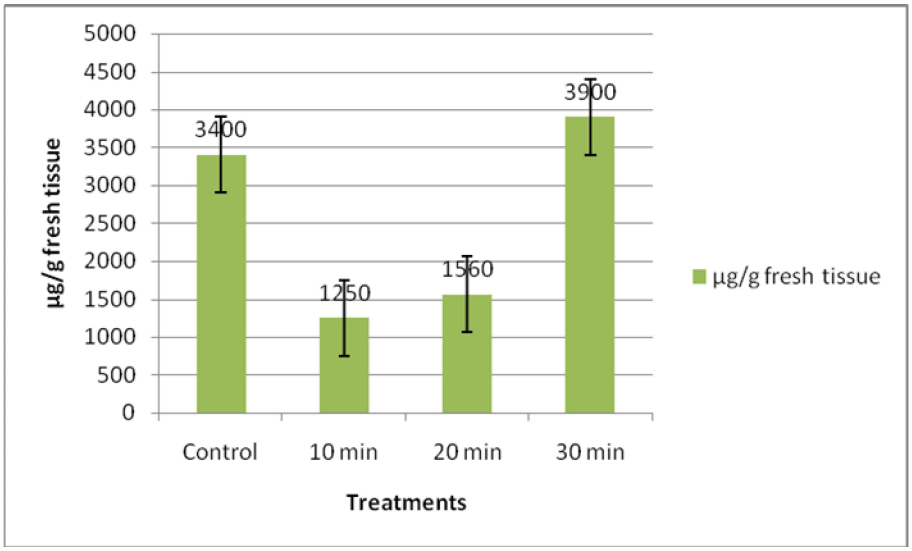


**Fig 6. Changes in the Protein content of black gram seedlings from heat treated seeds at 50$^{O}$C for different durations**

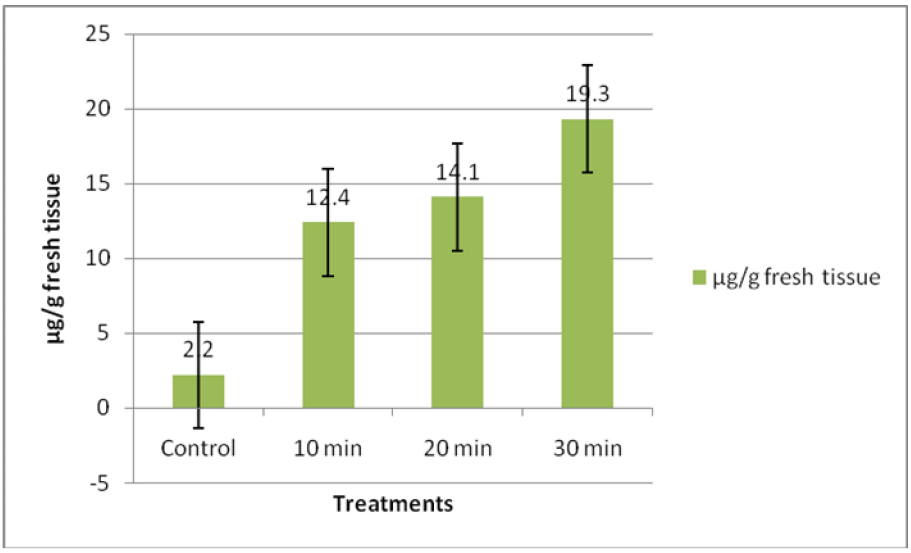


**Fig 7. Changes in the Proline content of black gram seedlings from heat treated seeds at 50$^{O}$C for different durations**

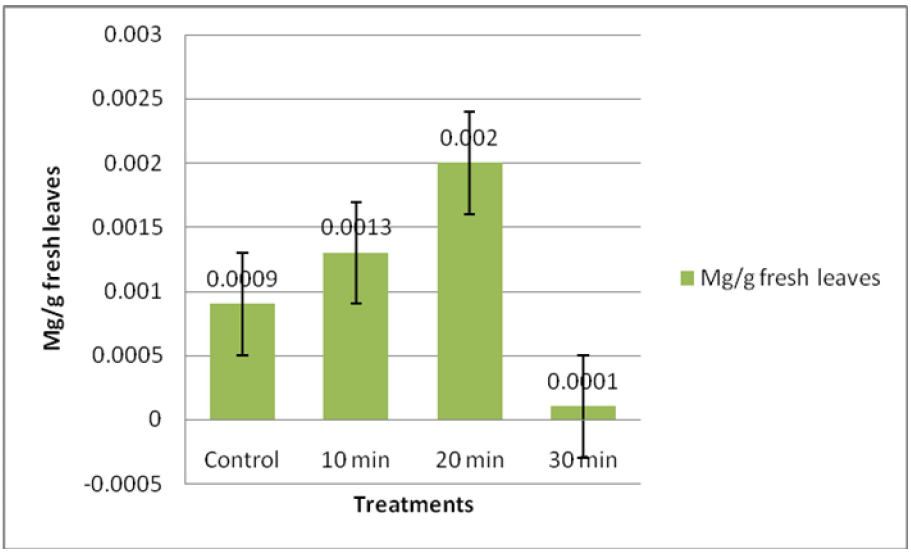


**Fig 8. Changes in the total Chlorophyll content of black gram seedlings from heat treated seeds at 50$^{O}$C for different durations**

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