

Study of the Exogenous Hormonal Regulation of Leaf Senescence in Two Millets, *Setaria italica*, L. and *Pennisetum typhoides*, Burm. 3. Study of Enzyme Activity-I (Catalase, Peroxidase and Polyphenyl Oxidase)

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Abstract: The present project is aimed at studying the exogenous regulation of leaf senescence in two millets i.e. *Setaria italica*, L. and *Pennisetum typhoides* Burm. The present study is confined to the study of excised leaf senescence by using growth regulators as the exogenous agents. Attached leaf study was avoided for the reason that preliminary experiments showed insensitiveness of the leaves of these two millets to growth regulators in the attached condition. In this study three growth regulators were selected, each from three major groups. Benzimidazole (BZI) represented cytokinins and indole acetic acid represented the auxins and GA₃ represented the Gibberellins. In the present investigation, the growth regulators had no effect on the catalase activity of *S. italica* and the effects were inconsistent in *P. typhoides*. Thus it appears that trends in catalase activity is species specific and cannot be generalized. The activity of peroxidase increased during senescence. There was some stimulation of peroxidase in *S. italica*. However in *P. typhoides* the enzyme was insensitive to exogenous agents. Thus peroxidase cannot show the extent of physiological decline in these plants. Polyphenol oxidase activity in *S. italica* exhibited a continuous increase up to the end of incubation both in dark and light treatments in contrast to *P. typhoides* where a 48 h fall was noticed.

Keywords: senescence, millets, hormones, enzymes

1. Introduction

Studies on senescence mostly covered plants like rice, wheat, maize and barley. In developing and developed regions of the world, millets (included under cereals) form an important group of plants in contributing to increased food production. As leaf senescence is considered as a physiological determinant of yield, it is the need of the day to understand the mechanism of leaf senescence and also the activities of enzyme involved in different metabolic processes to implicate the programmes aimed at increasing the crop productivity.

The role of enzymes cannot be overlooked in a process like senescence where decline in the levels of different macromolecules is striking, but a general theory is yet to be proposed regarding the role of enzymes involved in different metabolic activities. Senescence is mainly a catabolic process but some enzyme proteins are synthesized in an enormous rate even at the later stages of senescence. Two enzymes which utilize hydrogen peroxide are catalase and peroxidase. Catalase is considered as a reliable indicator of senescence (Patra et al., 1978) as there is a parallelism between chlorophyll loss and the changes in catalase activity in various monocot and dicot plants. However powerful anti senescence agents show opposite effects on chlorophyll content and catalase activity (Kumar and Khan, 1982). This makes the situation more complicated to draw any clear cut conclusion.

The involvement of various oxidative enzymes in senescence is well known and senescence is mainly an oxidative process. Compared to other reduced or active oxygen form, which act as oxidants in initiating leaf senescence, hydrogen peroxide, a reduced form of oxygen is the dominant metabolite in leaf tissue (Thompson *et al.*, 1987). Endogenous hydrogen peroxide levels increase during senescence (Brennan and Frenkel, 1977; Brady, 1988). Accelerated chlorophyll breakdown is observed in rice leaves when there is an exogenous supply of hydrogen peroxide or some substrates responsible for its synthesis (Parida et al., 1981).

Regarding the functional activity of peroxidase some reports say that the enzyme has a stabilising effect on chlorophyll (Birecka et al., 1979), whereas other reports claim that peroxide catalyzed hydrogen peroxide induced bleaching of chlorophyll (Huff, 1982). But it is not yet certain which is the *in vivo* substrate or hydrogen donor for peroxidase (Fric, 1976; Braber 1980). There are also evidences that monomeric peroxidase molecules are derived from tetrameric catalase molecules (Braber, 1980; Kumar and Khan, 1982). But concrete conclusions are yet to be drawn on the nature and role of these two enzymes. Poly phenoloxidase is an allied enzyme of peroxidase and both of them utilize phenols as their substrates. There are reports indicating increase in the activity of this enzyme during senescence (Farkas et al., Kisban et al., 1964; Maraitte, 1973; Kar and Mishra, 1976), but these reports are meager to ascertain the role of the enzyme.

2. Materials and Methods

Experimental Material

Graded seeds of *Pennisetum typhoides* Burm. And *Setaria italica* L were obtained from Agricultural Research station, Ratnapur in Ganjam district. The seeds were sown in small seed bed plots (1 X 1 m²). The plants were grown under natural conditions till the 4/5th day and the second leaves of these plants were used as the experimental material.

Selection of effective test chemical concentration

Leaves were collected, washed and randomized and floated in a range of concentration of each chemical both in the dark and under illumination. The chlorophyll content of these leaves was measured 48 h after incubation. From the texture of the leaves the toxicity of the chemical was assessed (loss of texture and development of dark patches was treated as a toxic response). Basing on the chlorophyll content of the leaves, the effective concentrations were chosen.

Three growth regulators, one from each major group (cytokinins, gibberellins and auxins), were tested for their efficiency in preventing chlorophyll loss. The optimum concentration of the cytokinin was 0.1 mM and IAA did not show any chlorophyll retention at very low concentration but with the increase in concentration, there was a corresponding rise in chlorophyll in leaves in the dark (up to certain concentration). The optimum concentrations chosen for both GA3 and IAA were 0.5 mM.

Incubation

The leaves were detached from the 6/7 day, old plants, randomized and leaf samples weighing ca 100 mg fresh weight were floated in petridishes (15 cm diameter) containing 50 ml solution of the test chemicals. As the experiments with the excised leaves were carried out both in the dark and under illuminated conditions, two sets of petridishes were incubated separately in the dark or under illumination (20 W m⁻²) at RT under aseptic conditions. Leaves floated in distilled water served as controls for both dark and light.

Selection of effective light Intensity

Visual changes were observed in the leaves within 24 h of floating in the dark. As the lower intensities were less effective and the higher ones possibly caused photo-bleaching effects, 20 W m⁻² was chosen as the optimum intensity for further experiments.

Excised leaves were collected, washed, randomized and leaf samples weighing ca 200 mg fresh weight were floated in petridishes containing distilled water. The petridishes were incubated in the dark or under different intensities of continuous light (10, 20, 30, 40, 50, 70, 100, 200 W m⁻²). For the purpose of providing different light intensities, white light from TL 40W/54 fluorescent lamps of Philips (India) along with 25W incandescent lamp were used and the chlorophyll content was measured 48 h after incubation.

Estimation of Enzyme activity:

The enzyme activities (Catalase, Peroxidase and Polyphenyl oxidase) of hormone treated leaves (both under dark and

illumination) were estimated following the procedures of Kar and Mishra (1976).

Statistical treatment:

For all the data, deviations from means have been indicated in the form of standard errors. In a number of graphs, the points plotted are either overlapping or remain very close, hence, the standard error values have not been shown in the graph to avoid clumsiness and confusion. Wherever necessary, student's-t-test were performed.

3. Results

The results obtained after study of Effect of growth regulators in the dark and under illumination on enzyme activities in the leaves of *P.typhoides* and *S.italica* are given in Fig. No. 01-12.

Both the dark incubated and the illuminated leaves exhibited a slow and gradual increase in the catalase enzyme activity with time. The enzyme activity in both the cases was 54% more than the initial activity by the end of 96 h.

Peroxidase activity increased with time both in the dark incubated and illuminated leaves. However there was a post 72 h fall in both the cases. At 96 h the dark incubated leaves exhibited an increase of 47.5% over initial peroxidase enzyme activity compared to 76.4% increase in the corresponding illuminated controls proving that light had an inducing action on the enzyme activity.

A steep rise in the polyphenol oxidase enzyme activity with time was well noticed in both dark incubated and illuminated leaves. The rise was almost parallel at different stages of incubation in both the conditions.

4. Effect of growth regulators in the dark

All the growth regulators exhibited almost a parallel rise in catalase enzyme activity at all the stages of incubation causing an increase of 53-56% initial catalase enzyme activity at the end of incubation.

Growth regulators caused a significant increase in the enzyme activity in the dark. However a mild post 72 h fall in the enzyme activity was observed in IAA and GA3 treated leaves. In these treatments the increase at 96 h was 69.7% and 93.2% respectively of the initial activity. A gradual increase in the Peroxidase activity was observed in the BZI treated leaves showing an increase of 94.7% over the initial activity at the end of incubation. This proves BZI and GA3 to be equally efficient in causing the enzyme activity retention.

Growth regulators increased further the enzyme activity with time. BZI and GA3 were similar in their action in causing enzyme activity retention. At the end of the experiment both of them caused 285.8% of initial enzyme activity. IAA was slightly mild causing 263% of initial enzyme activity at 96 h.

Effect of growth regulators under illumination

Light had no specific effect on the catalase enzyme activity even in the presence of growth regulators as the growth regulator treatments under illumination showed an almost similar enzyme activity during all stages of incubation like the dark incubated leaves.

A gradual increase up to 72 h and thereafter a post 72 h fall identical with the dark incubated leaves was clearly evident in all the growth regulator treated leaves. GA3 was most efficient in which the enzyme activity retention was 82.6% more than the initial enzyme activity. BZI and IAA were equally efficient and the enzyme activity in these growth regulator treatments were almost similar with the corresponding controls at 96 h showing an increase of 74-78% over the initial enzyme activity

Similar to the dark incubated leaves, the illuminated leaves in response to growth regulator treatments exhibited an additional increase in enzyme activity. GA3 was the most efficient among all the growth regulators causing 302.1% of initial enzyme activity at the end of the experiment. BZI could increase the enzyme activity to 193.4% more than the initial activity at the same time. IAA was least effective in which at 96 h an increase of 166.2% over the initial enzyme activity was observed.

5. Discussion

The involvement of various oxidative enzymes in senescence is well known and senescence is mainly an oxidative process. Compared to other reduced or active oxygen form, which act as oxidants in initiating leaf senescence, hydrogen peroxide, a reduced form of oxygen is the dominant metabolite in leaf tissue (Thompson *et al.*, 1987). Endogenous hydrogen peroxide levels increase during senescence (Brennan and Frenkel, 1977; Brady, 1988). Accelerated chlorophyll breakdown is observed in rice leaves when there is an exogenous supply of hydrogen peroxide or some substrates responsible for its synthesis (Parida *et al.*, 1981).

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Thus it appears that trends in catalase activity is species specific and cannot be generalized.

The activity of peroxidase increased during senescence. There was some stimulation of peroxidase in *S. italica*. However *P. typhoides* the enzyme was insensitive to exogenous agents. Thus peroxidase cannot show the extent of physiological decline in these plants.

Polyphenol oxidase activity in *S. italica* exhibited a continuous increase up to the end of incubation both in dark and light treatments in contrast to *P. typhoides* where a 48 h fall was noticed.

In the present investigation, the cytokinin, BZI was found to be effective in arresting the senescence process in the leaves of both *P. typhoides* and *S. italica* in the dark and in the light. *Pennisetum* was relatively more sensitive to BZI compared to *Setaria*. This observation corroborates the reports that cytokinins show universality in their anti senescence properties (Miller, 1961; Skoog and Armstrong, 1970; Kende, 1971; Hall, 1973; Thomas and Stoddart, 1980; Goldthwaite, 1987; Nooden, 1988; Van Staden *et al.* 1988).

The mechanism of hormone action is extensively studied and a number of reviews have been published that are related to the hormonal regulation of senescence (Cherry, 1967; Sacher, 1967; Wollgiehn, 1967; Woolhouse, 1967; Beevers, 1976; Letham, 1978; Leopold, 1980; Nooden, 1980; Thimann, 1980, Thomas and Stoddart, 1980; Sabater, 1985; Nooden and Letham, 1986; Goldthwaite, 1987; Brady, 1988).

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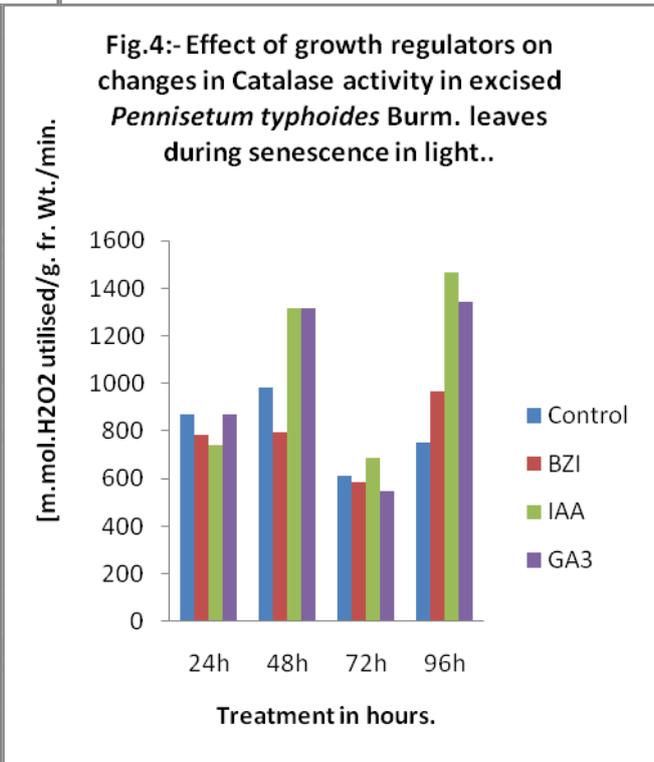
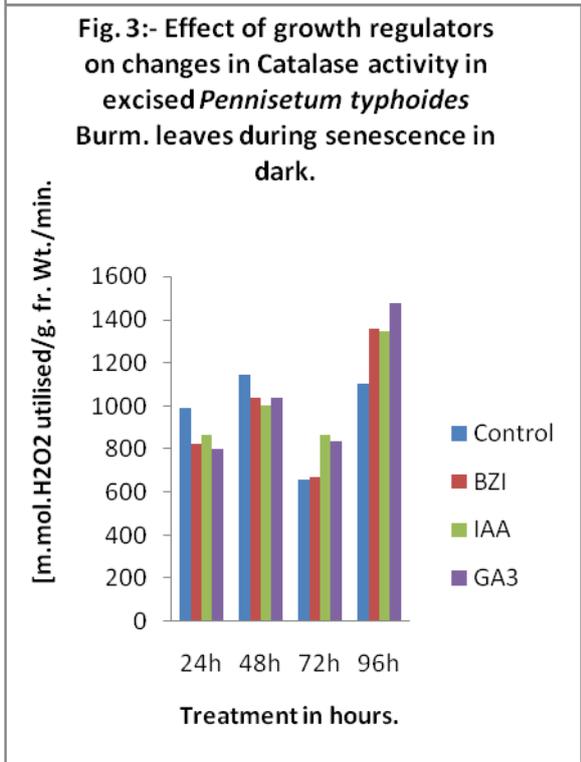
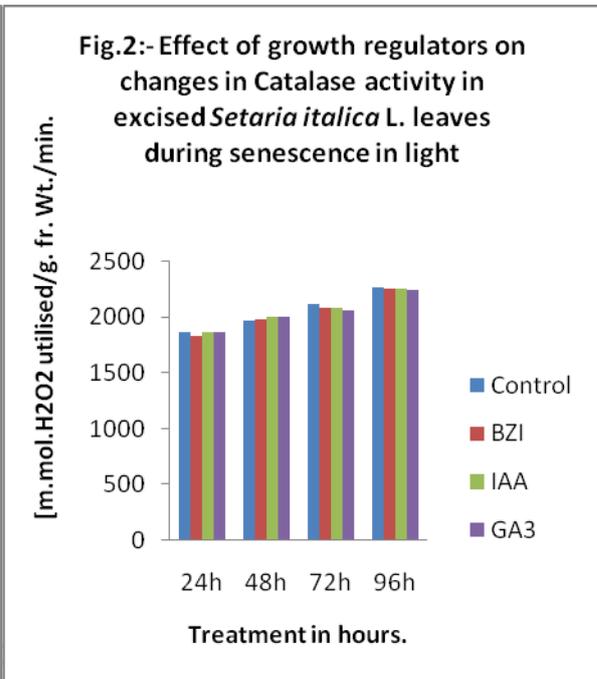
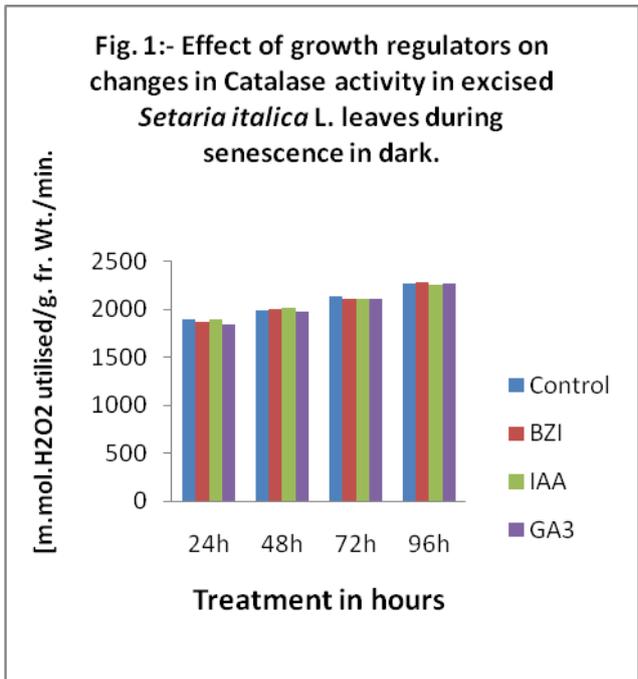


Fig.5:-Effect of growth regulators on the changes in the peroxidase activity of excised leaves of *Setaria italica* L. during senescence in dark.

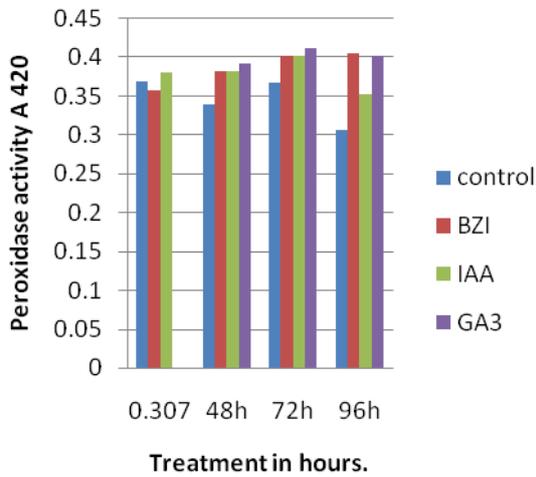


Fig 6: .Effect of growth regulators on the changes in the peroxidase activity of excised leaves of *Setaria italica* L. leaves during senescence in light.

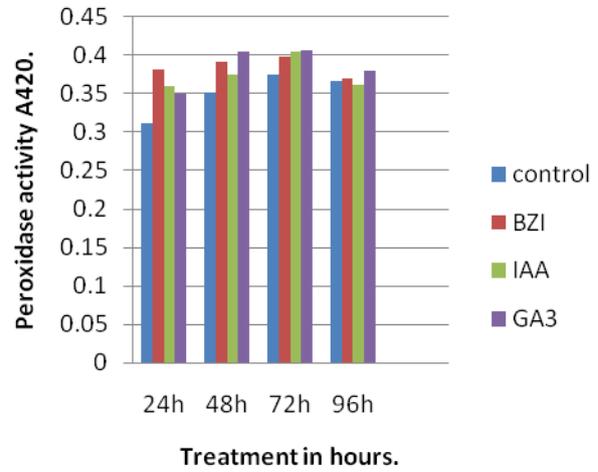


Fig. 7 :-Effect of growth regulators on the changes in the Peroxidase activity of excised leaves of *Pennisetum typhoides* Burm.in dark

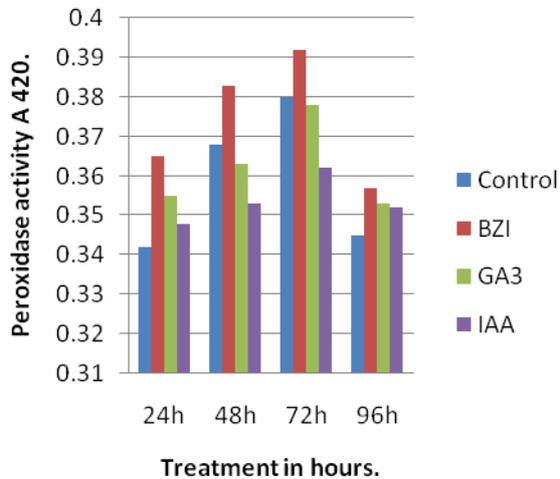


Fig 8:-Effect of growth regulators on the changes in the Peroxidase activity of excised leaves of *Pennisetum typhoides*. Burm. Leaves during senescence in light.

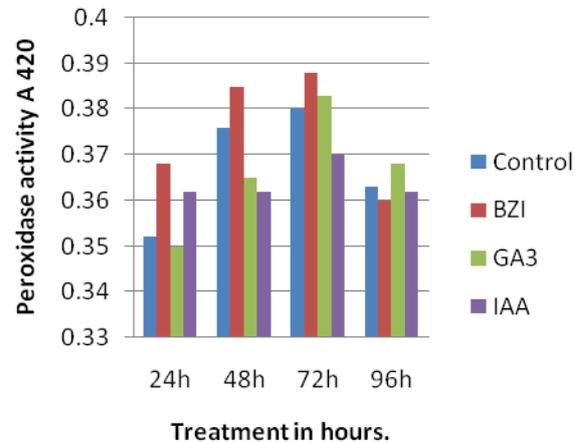


Fig.9 :-Effect of growth regulators on the changes in the Polyphenol oxidase activity of excised leaves of *Setaria italica*. L. during senescence in dark.

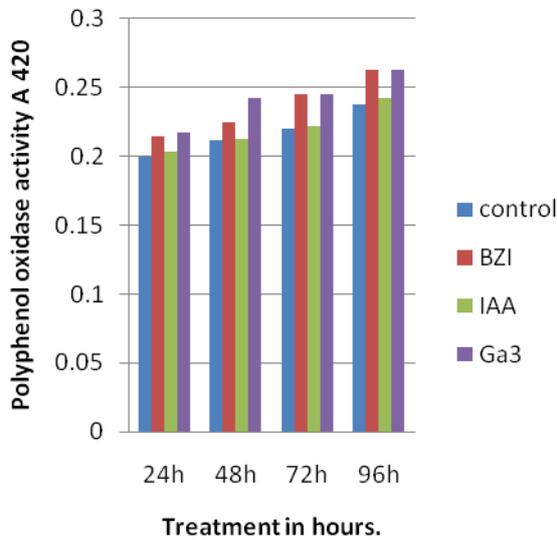


Fig. 10:-Effect of growth regulators on the changes in the Polyphenol oxidase activity of excised leaves of *Setaria italica*.L.leaves during senescence in light.

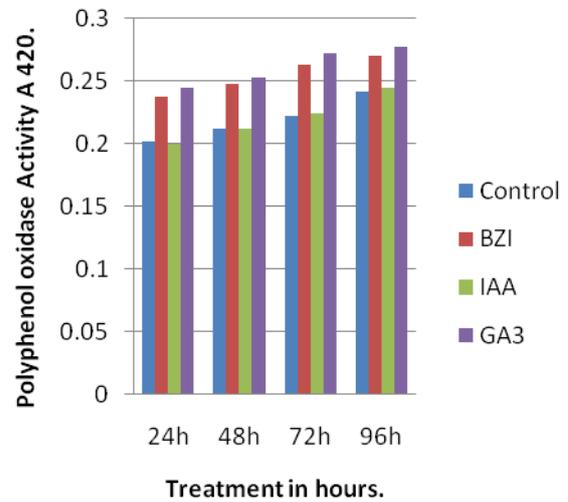


Fig.11:-Effect of growth regulators on the changes in the Polyphenol oxidase activity of excised leaves of *Pennisetum typhoides* Burm. Leaves during senescence in dark.

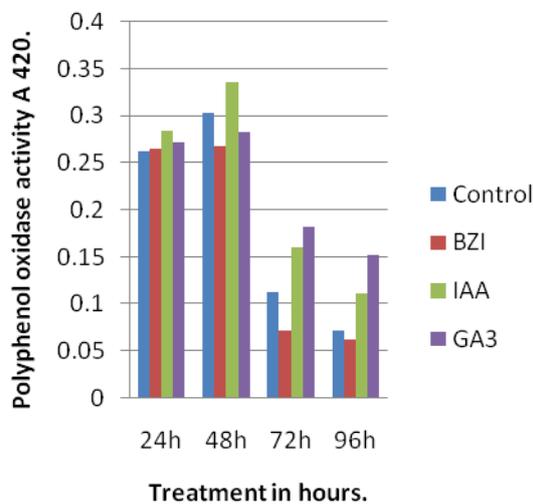


Fig. 12:-Effect of growth regulators on the changes in the Polyphenol oxidase activity of excised leaves of *Pennisetum typhoides* Burm. Leaves during senescence in light.

