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Research Article

Impact of some phytohormones, metabolic inhibitor and agrochemicals on senescing paddy leaves and its relevance to productivity

Haseena Rafath
Department of Botany, Government Arts College, Mutlur, Chidambaram, Tamil Nadu, India.

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Abstract: The influence of hormones viz. Indole acetic acid (IAA), Gibberellic acid (GA_3), Cytokinins viz. Benzyladenine (BA), Benzimidazole (BZI), biocides viz. 2,4-Dichlorophenoxy acetic acid (2,4-D), Diathane M-45, Dimecron and metabolic inhibitors viz. Cycloheximide (CHI) and Actinomycin-D (Act-D) on senescence in detached paddy (*Oryza sativa* L.) leaves was studied under dark and illuminated conditions. Cytokinins were found to be more effective senescence retardants in light than in dark while auxins were effective in light. Biocides proved to be milder senescence retardants. Effective concentration, a concentration away from the ineffective and toxic range, chosen from IAA, GA_3, BA, BZI, 2,4-D, Diathane M-45 and Dimecron were 25,100, 5, 100, 25, 1500 and 0.50 ppm respectively. Application of Act-D (inhibitor of mRNA synthesis) with in 15 min after detachment and cycloheximide (inhibitor of cytoplasmic protein synthesis) from the start of the incubation resulted in suppression of peroxidase activity both under light and dark. Catalase activity exhibited an inverse relation with peroxidase activity under both the experimental conditions. It is recommended that attempts must be made in a more sophisticated way to study the changes in activities of different enzymes suspected to be linked with leaf senescence at a deeper level using all modern techniques available so that the exact nature of enzymes, the isoenzymes pattern and their relation with yield can be understood. Such studies may also help in future in manipulating the projects for the improvement of this economically important cereal crop.

Key words: *Oryza sativa L.* Senescence; auxins; cytokinins; biocides; metabolic inhibitors; catalase; peroxidase.

Introduction

Leaf senescence is a complicated process controlled by a number of intrinsic and external factors. Study of leaf senescence has been mainly grouped into two categories (a) attached leaves (leaves attached to plant) and (b) detached or excised leaves (leaves detached from plant). Most of the investigators (Beevers, 1976; Nooden, 1980; Thimann, 1980, 1985, 1988), studied senescence in excised organs such as leaves, cotyledons, fruits and so forth. While such systems may be easier to handle experimentally, it is by no means certain that the results observed with excised plant materials bear directly on the question of senescence. Interesting and valuable results have been observed with excised organs which correlated with the results from intact organs. The literature on leaf senescence has been reviewed by Woolhouse (1974, 1982, 1984) and Thimann *et al.,* (1982), Thimann (1988). Change in colour of the leaf from green to yellow due to loss or break down of chlorophyll and changes in activity of catalase and peroxidase enzymes are treated as reliable indicators of senescence.

Leaf senescence in agriculturally important plants is considered as a physiological determinant of yield. Here understanding of leaf senescence is essential to implicate the programs viewed at increasing crop productivity. Since, senescence is an inevitable event in the life cycle of a living organism, there developed an urge to know the detailed sequence in the organ / plant and then a relative influence of a variety of chemicals and environmental factors on senescence and thereby through other investigations to find out if there is any environmental factor or a chemical of a particular strength to prevent or delay senescence so that the technique can be utilized for lengthening the life span of plants which have direct bearing on increase in production. It is the relation of yield to the length of period of retention of green colour of leaves in plant that attracted the attention of recent biologist's world over. The study of senescence, thus, assumed greater importance in agriculture. There has been an extensive use of chemicals to increase yield. It is natural to expect that a chemical used for inducing a particular physiological process may have its effect on other processes such as retention or degradation (break down) of chlorophyll which has relation to yield. Biocides are extensively used in modern agriculture to augment production. Hence, a large number of these biocides are flooded into the market for use in agriculture by farmers. Therefore, there is a need to know if these herbicides while facilitating better growth of crop plants would otherwise cause any deleterious effect on the longevity of crop plants.

***Corresponding Author:**
Dr. Haseena Rafath,
Department of Botany, Govt Arts College,
Mutlur, Chidambaram, Tamil Nadu, India.
E-mail: rahamtullah1967@yahoo.com

Photoperiod is considered to be one of the most effective environmental factors affecting the plant's life. Light intensity, lower or greater than the minimum may alter the rate of photosynthesis effecting the quantum of production as photosynthesis has relation to yield in paddy. Therefore, the present study was aimed at identifying the relative influence of light intensity and different concentrations of auxins, cytokinins, one representative of each of the three categories of biocides (fungicide, pesticide and herbicide) as well as metabolic inhibitors on senescence in detached paddy leaves.

Materials and Methods

Uniform, healthy and well expanded green leaves excised from 30-day-old paddy (*Oryza sativa* L. cv. Ratna) plants were used as experimental material for the present investigation. Raising nursery from pure line seeds of paddy was accomplished following procedure adopted by Khan *et al.,* (1979). Aqueous solutions of 5, 10, 25, 50, 100, 250 and 500 ppm strength each of the two auxins viz. Indole Acetic Acid (IAA), Gibberellic acid (GA_3), two Cytokinins viz. Benzyladenine (BA), Benzimidazole (BZI), three biocides viz. 2,4-Dichlorophenoxy acetic acid (2,4-D, herbicide), 500, 1000, 1500, 2000 and 2,500 ppm strength of Diathane M-45 (fungicide), 0.10, 0.25, 0.50, 100 and 1.50 ppm strength of Dimecron (insecticide) were prepared with double glass distilled water. Metabolic inhibitors viz. Cycloheximide (CHI, inhibitor of cytoplasmic protein synthesis) and Actinomycin-D) (Act-D, inhibitor of mRNA synthesis) were also used. Excised leaf samples weighing ca 200 mg fresh weight were collected, washed and randomized and were floated in separate petridishes lined with filter paper moistened with a measured quantity (50 ml) of double glass distilled water or test solution. A small but a requisite concentration (20 mg/l) of antimicrobial and antifungal agent, streptomycin sulphate was also taken in petridishes for arresting microbial growth (De Leo and Sacher, 1970) and then incubated in fluorescent light (2000 lux) from TL 40W/54 fluorescent lamp of Philips (India) or dark stress. Continuous white light ca 200, 500, 100, 2000, 3000 and 5000 lux was used to determine the optimum intensity of light required to keep the photosynthetic activity at optimum level in paddy leaves as light intensity lower or greater than the optimum may alter the rate of photosynthesis effecting the quantum of production. Continuous white light and its intensity in terms of lux was measured at the level of floating excised leaves with the help of lux meter. In order to maintain aseptic conditions the experimental chambers were subjected to UV irradiation and were washed with 95% ethanol prior to incubation. All the experiments were performed in sterilized Corning glassware. Petridishes each containing 5 replicates for each concentration of each test chemical were incubated in dark at 28°C±2°C. A separate set of petridishes containing 5 replicates for each concentration of each of the test chemicals were incubated under continuous illumination of white light of 2000 lux intensity. There was a control set for both light and dark-incubated leaves. Care was taken that all experimental sets remaining at equal distance from the light source. In all the treatments, the pH of the test solution as always adjusted to neutrality with the help of 0.1 (N) NaOH. Time taken for complete change in leaf colour from green to yellow was recorded and was taken as the criterion of senescence. Catalase and Peroxidase enzymes, also considered as reliable senescence indicators, were estimated. The activity of catalase was assayed following the method of Snell and Snell (1971) with some modifications as followed by Biswas and Choudhuri (1978). The enzyme activity was expressed as enzyme unit $min^{-1}g^{-1}$ fr. wt. Peroxidase activity was determined by following the rate of oxidation of pyrogallol (Chance and Maehly; 1955) as described by Kar and Mishra (1976) and its activity was expressed as M mole purpurogallin formed g^{-1} fr wt min^{-1}.

Results and Discussion

Visible change in natural colour of the leaves through transitional colours, to yellow occurred centrifugally in excised paddy leaves kept in dark or continuous illumination in petridishes containing distilled water or test chemical. The number of days required for complete changeover of leaf colour from green to yellow was recorded in each treated set.

Leaves incubated in dark and low intensity of 200 lux senesced in two days. With an increase in the intensity of light, there was a proportionate delay in senescence period. Thus, excised paddy leaves took 2.50, 2.80, 3.60 and 4.20 days to senesce in light of 200, 500, 1000 and 2000 lux respectively. Further increase in the light intensity resulted in a gradual fall in the senescence period as excised leaves subjected to continuous light intensity of 3000 and 5000 lux senesced in 3.80 and 3.60 days respectively (Fig. 1). The effective intensity of light was between 1000 to 3000 lux. However, for convenience and safety 2000 lux was treated as the effective intensity of light. Thus, light is an efficient leaf senescence retardant and its effectiveness in bringing out senescence retardation had been proved with different cereal plants like Oat (Thimann *et al.,* 1977), ragi (Kumar and Khan, 1982a) and other plants like bean, (Goldthwaite and Laech, 1967; Chen, 1972), Hibiscus (Mishra and Biswal, 1973) and soybean (Hsia and Kao, 1977). The present study adds one more plant to the list where light intensity of 2000 lux acted as a powerful senescence retardant. Hsia and Kao (1977), Thimann *et al.,* (1977), however, reported phototoxicity at an intensity of 1000 lux or more in oat leaves. In paddy

leaves an intensity of 4000 lux or more induced phototoxic effects. The difference in the behaviour of oats and paddy leaves may be due to leaf age and plant species.

Selection of effective concentration of different chemicals on senescence period

Indole acetic acid markedly delayed senescence of excised paddy leaves incubated under continuous light over those incubated in dark at each concentration tried. IAA at low concentration of 5, 10 and 25 ppm caused a marked delay in senescence under both the conditions. With an increase in the strength of the chemical there was an increase in the period for the excised leaves to senesce. Further increase in the chemical strength from 50 to 250 ppm hastened senescence, while higher concentration of 500 ppm proved to be toxic as it increased to the maximum the green and brown patches which were minimum in senescing leaves at lower concentration of IAA (Fig. 1). Gibberllic acid too significantly caused senescence inhibition, it being more marked with increasing concentration of the chemical. Thus, the period required by excised leaves to senesce ranged from 5.40 days at 5 ppm to 8.50 days at 100 ppm as against those incubated in dark where the senescence period ranged for 4.30 days at 5 ppm to 6.45 days at 100 ppm concentration. Further increase in the concentration of GA_3 hastened senescence both in light and dark condition. The chemical at higher concentration of 500 ppm was toxic. GA_3 at a concentration of 100 ppm was the most effective one in inhibiting senescence (Fig. 1).

Benzyladenine was effective at low concentration of 5 ppm in delaying senescence both light and dark-incubated leaves compared to their respective controls in light incubated leaves; the hormone delayed senescence for 2 days over the control at 5 ppm. Further increase in the concentration (10, 25 ppm) resulted in a gradual fall in the senescence period. Similar was the trend in leaves incubated in dark, but the magnitude of difference in senescence period being more in light- incubated leaves than dark-incubated ones at each concentration (Fig. 1). The chemical of 50 ppm strength or higher caused toxic effects, but the toxic effects were different from the effects caused by IAA. In this case, the leaves had no brown patches. They had only dark green patches which increased with an increase in concentration and there was also a marked change in the texture of the leaves as leaves became soft and brittle similar toxic effects were also noticed in dark-incubated leaves treated with higher concentrations (50 ppm or more) (Fig. 1). BZI, like BA delayed senescence period both in light and dark conditions. Under illumination, the efficiency of BZI increased with an increase in concentration and the chemical delayed senescence of the excised paddy leaves by 2,3,4,5 and 6 days over the light controls at the concentration of 5,10,25,50 and 100 ppm respectively. The chemical at 250 and 500 ppm hastened senescence and the leaves exhibited a mild change in texture. The leaves floated at concentration of 5, 10, 25, 50, 100, 250 and 500 ppm senesced more rapidly than their respective sets incubated in light. From a concentration of 5 ppm onward the efficiency of BZI in retarding senescence increased and reached optimum level at 100 ppm and remained constant with lesser period of senescence at higher concentration of 250 and 500 ppm (Fig. 1).

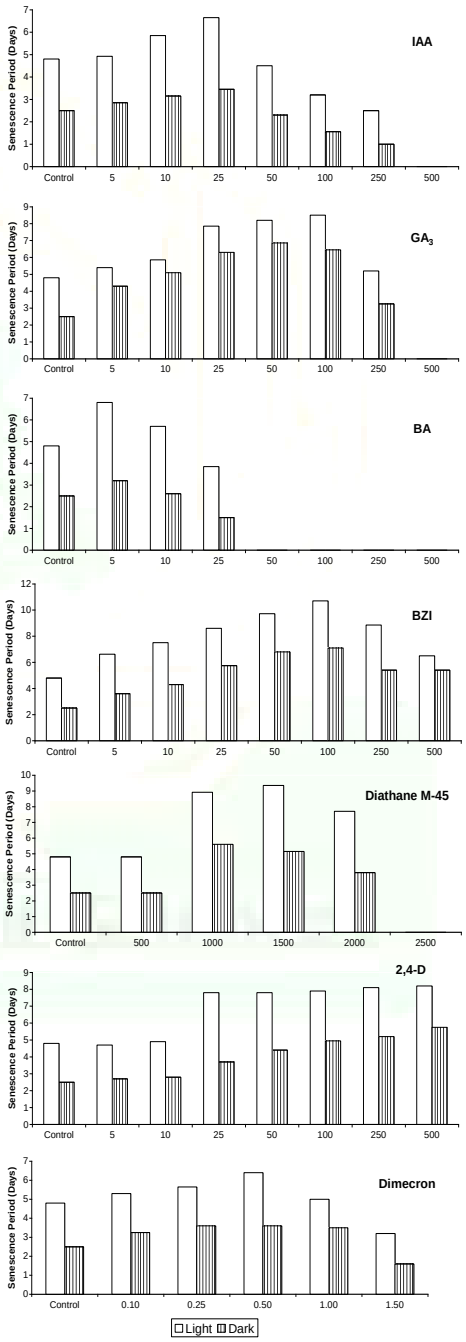


Figure 1: Effect of different chemicals on senescence period in excised paddy leaves during senescence

The finding of kinetin as senescence retardant in *Xanthium* by Richmond and Lang (1957) set in motion the studies on the hormonal regulation of senescence. Of several synthetic and natural growth regulators having senescence retarding properties, cytokinins got the maximum attention due to their universality in application (Skoog and Armstrong, 1970; Kende, 1971). Auxins (IAA, GA_3) at lower concentrations were ineffective in retarding senescence and higher concentrations retarded senescence in detached paddy leaves though not as efficiently as cytokinins (Kao, 1978). Cytokinins were reported to be effective in the cereals like rice (Mishra and Misra. 1973; Kao. 1978; Khan *et al.,* 1978), ragi (Khan *et al.,* 1979), wheat (Person *et al.,* 1957; Waygood, 1965) and oat (Gunning and Berkley, 1963; Verga and Bruinsma, 1973; Biddington and Thomas, 1978). The findings in the present study fully agree with the results of the previous workers cited. BA and BZI applied to paddy leaves in the present study were more effective at all concentrations tried in light than in dark, an observation which differs from Engelbrecht (1971) who observed little of kinetin on leaves exposed to light In the present study, marked senescence retardation in excised paddy leaves by lower concentration (5 ppm) of purine cytokinin (BA), IAA (25 ppm) and higher concentration of BZI and GA_3 (100 ppm) was observed and thus supports the reason why invariably in all senescence experiments with different plants, using cytokinins, lower concentrations of kinetin and BA and higher concentrations of BZI have been used (Singh and Mishra, 1975; Mishra and Misra, 1973; Mishra and Pradhan, 1973; Kao, 1978). Higher than the effective concentrations of growth regulators augmented senescence, a finding which concurs similar observations made by Misra and Das (1978) in *Cestrum nocturnum* L.

2,4-Dichlorophenoxy acetic acid at low concentrations of 5 or 10 ppm did not produce any significant effect on senescence period in excised paddy leaves incubated either in light or dark as compared to their respective control ones; but at higher concentrations (25 ppm) it brought about a significant inhibition in the senescence proportionate to the strength of the chemical in dark condition, while the leaves incubated in the light exhibited the maximum of 3 days in the leaves treated with 25 ppm of 2, 4-D and higher concentrations did not cause marked difference in the senescence period which remained more or less constant. Thus, 2,4-D acted as a milder senescence inhibitor than BA or BZI (Fig. 1).

Diathane M-45 at lower concentration of 500 ppm has no marked effect on senescence period of excised paddy leaves incubated either in light or dark in comparison with the controls. At higher concentration of 100 and 1500 ppm there was a delay in senescence period of excised leaves proportionate to the chemical strength under both the conditions, the delay being greater in light than in dark condition. Further increase in the strength of the chemical caused a decrease in senescence period in both light and dark incubated leaves (Fig. 1). The highest concentration (2500 ppm) tried had toxic effect. Dimecron at low concentrations of 0.10, 0.25 and 0.50 ppm brought about a marked delay in senescence period proportionate to the strength of chemical both in light and dark condition. An increase in concentration of the chemical beyond 0. 50 ppm resulted in a gradual fall in the senescence period. The increase or decrease in senescence period in treated leaves was greater in light rather than in dark condition as compared to their respective controls (Fig. 1). In conclusion, it appears that both light and cytokinins are more powerful senescence retardants in paddy leaves. Cytokinins are more effective in light than in dark. Auxins (IAA and GA_3) are more effective only in light. Biocides (2,4-D, Diathane M-45 and Dimecron) are mild senescence retardants. Diathane M-45 was more effective senescence retardant than the rest of the two biocides. The effective concentrations chosen from the effective range of IAA, GA_3, BA, BZI, 2,4-D, Diathane M-45 and Dimecron were 25, 100, 5, 100, 25, 1500 and 0.50 ppm respectively as these are away from the ineffective and toxic range. Biocides, like hormonal chemicals, were more effective in light than in dark. Lower or higher than the recommended concentrations of these chemicals were ineffective in retardation of chlorophyll degradation. Diathane M-45 at 1500 ppm was more effective senescence retardant than 2,4-D (25 ppm) or Dimercon (0.50) in excised paddy leaves. The data of the present study support the similar findings reported earlier by Kumar and Khan (1982a) in ragi leaves, Patro (1984), Ranjani (1985) in rice leaves.

Catalase and peroxidase as indicators of senescence

In light, catalase activity exhibited a mild increase following an increase in the incubation period, while in dark the catalase activity was considerably lower. Growth regulators, in general, enhanced catalase activity which declined following an increase in the incubation period in both the conditions, the magnitude of increase being more in light than in dark. IAA and GA_3 did not exhibit much difference in their effect on catalase activity which was more pronounced in BA and BZI-treated ones, BA being more effective than BZI. Catalase activity was much less in leaves treated with biocides among which Dimecron was more effective than Diathane M-45 and 2,4-D was the least effective chemical. In dark, all the chemicals proved to be less effective than their counter parts in light (Fig. 2). In light and dark, the peroxidase activity exhibited a reverse trend to the catalase activity. In general, peroxidase activity exhibited an inverse relationship with catalase

activity. An increase in catalase activity was accompanied by a decrease in peroxidase activity or vice versa. The relative effectiveness of different chemicals on peroxidase activity both in light and dark condition exhibited a trend similar to the one noticed with respect to catalase activity. All the chemicals are equally effective in rising peroxidase activity (Fig. 3).

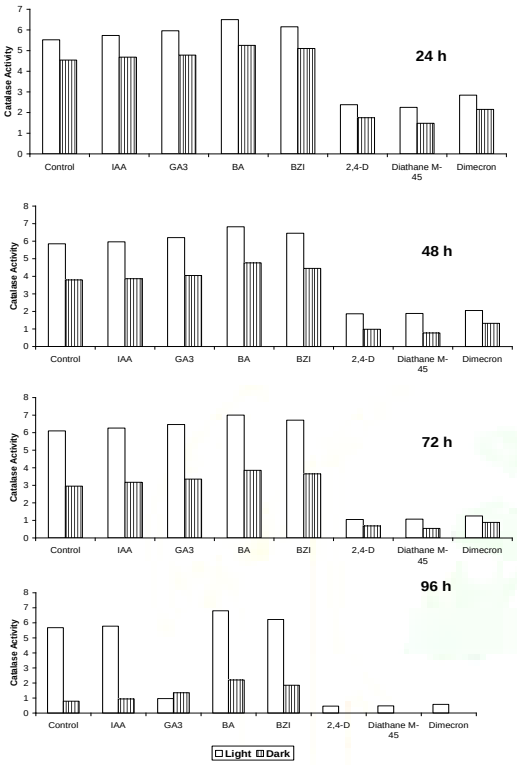
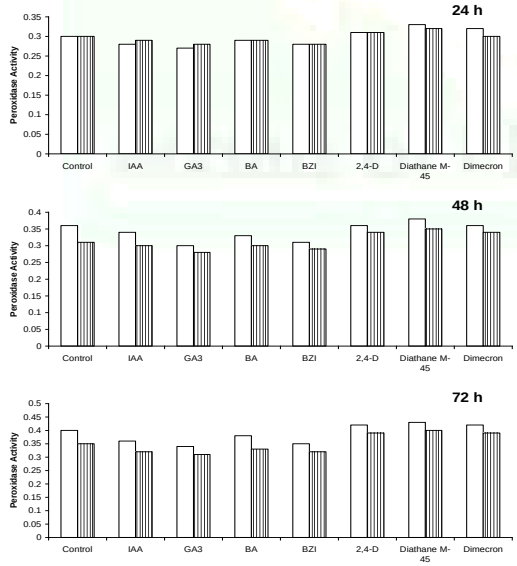


Figure 2: Changes in catalase activity in senescing excised paddy leaves as influenced by different chemicals in light and dark.



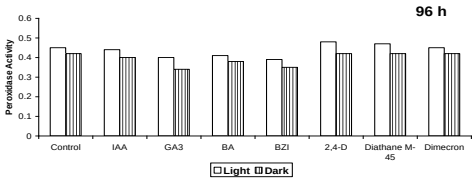


Figure 3: Changes in peroxidase activity in senescing excised paddy leaves as influenced by different chemicals in light and dark.

Effect of CHI and Act-D on catalase and peroxidase activity of excised paddy leaves during senescence

Application of 30 µM cycloheximide to excised paddy leaves caused a decrease in the peroxidase activity both in dark and light-incubated senescing paddy leaves. However, the inhibitor could check the rise in enzyme activity only partially. Act-D had no effect on the peroxidase activity of detached senescing paddy leaves (Fig.3). Catalase activity was also sensitive to cycloheximide. Like any growth regulator or light, cycloheximide caused a marked check in the decline of catalase activity in light and dark-incubated excised paddy leaves. As in case of peroxidase, application of Act-D had no effect on the activity of catalase in excised leaves incubated in light and dark (Fig. 4).

Changes in Catalase activity

In the present study, there was a rise in catalase activity more in light than in dark. Hormonal chemicals which acted as senescence retardants depressed the catalase activity. Biocides too decreased the activity of this enzyme. In fact, catalase and peroxidase are two peroxisomal enzymes responsible for the removal of hydrogen peroxide from the plant tissues. An exogenous application of H_2O_2 enhances the process of senescence. It is believed that the role of the two enzymes is very important in regulating the peroxidase levels during senescence.

Involvement of catalase in senescence has been quite controversial subject Earlier workers have mostly concentrated on the activity of catalase in excised leaves during senescence. The information so far available indicated that the excised senescencing leaves incubated in dark exhibited a decreased catalase activity in rice leaves (Kar and Mishra, 1976; Patro *et al.,* 1978), ragi leaves (Reddy *et al.,* 1985). *Anagalli leaves* (Trippi *et al.,* 1980). Henry and Jordan (1977) reported depression in catalase activity in excised soybean leaves treated with biocides. Such opposite trends in catalase activity observed in different plants may be due to species specificity. Catalase is believed to break down H_2O_2 evolved in photorespiration and maintains low levels of the toxic hydrogen peroxide. With an onset of senescence, there is a decrease in catalase activity and peroxide accumulation dominates and thus causes rapid senescence.

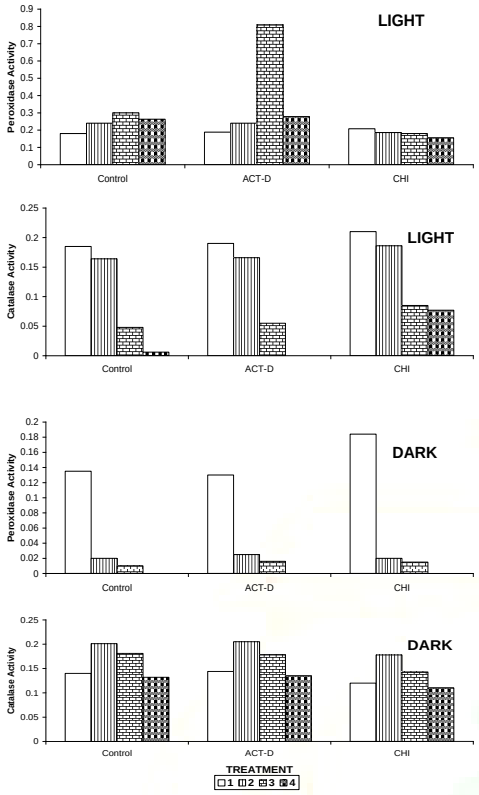


Fig. 4: Changes in peroxidase and catalase activity in senescing excised paddy leaves as influenced by metabolic inhibitors. 1,2,3 and 4 represent the number of days of incubation.

Changes in Peroxidase activity

In the present study, a rise in peroxidase activity was observed in excised senescing paddy leaves incubated in light and other senescence retarding chemicals like BA, BZI and 2,4-D. This concurs similar findings reported in detached rice leaves (Kar and Mishra, 1976; Patra *et al.,* 1979; Reddy *et al.,* 1985), tobacco (Parish, 1968) and oat (Birecka *et al.,* 1979; Kar and Feieraband, 1984). There are also reports of suppression of peroxidase activity by growth regulators in barley leaves (Sharma and Biswal, 1976) and pea stem sections (Henry and Jordan, 1977). A sharp rise in peroxidase activity in light-incubated paddy leaves indicates that this isozyme is light-dependent and is influenced by light. Such light-dependence of this enzyme was also observed by Birecka *et al.,* (1979) in oat leaves. Accumulation of oxy-free radicals following light enhanced peroxidase activity in light (Kar and Feieraband, 1984) and accelerated chlorophyll bleaching (Huff, 1982) clearly point out that peroxidase isozymes play an important role in leaf senescence. Samejima *et al.,* (1968) suggested that tetrameric catalase molecules break to form monomeric peroxidase molecules resulting in several folds increase in peroxidase during senescence. However, peroxidase activity decreased in dark-incubated leaves and was relatively higher under light; whereas catalase exhibited a reverse condition, namely a rise in dark and fall in light thus suggesting the possibility that a rise in peroxidase is due to the breakdown of catalase molecules (Kumar and Khan, 1982b, 1983). Though the functional role of enzyme is not yet clearly known, peroxidase is considered as a reliable indicator of senescence (Parish, 1968; Ford and Simon, 1972; Kar and Mishra, 1976; Kumar and Khan, 1982, 1983). A much deeper probe is necessary before ascribing a scavenger's role (induction of chlorophyll bleaching) to the enzyme (Huff, 1982) in the senescence process. A more comprehensive study is required on this /controversial enzyme to understand and ascertain its functional significance by knowing the *in vivo* source of substrate and hydrogen donor. A careful and intensive study with a variety of plants using all chemicals normally used in agriculture and other physiological studies is required to understand this complex phenomenon of senescence.

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