

Effect of Thiourea on the Germination, Respiration and Growth
of Amaranthus chlorostachys Seeds.

By

A.E. Dewidar

S.H. Rabie

Biology Department, Faculty of Education
Ain-Shams University, Cairo, Egypt.

Abstract:- The effect of thiourea on the germination, respiration and growth of Amaranthus chlorostachys seeds is presented. It is suggested that thiourea may act on the seeds by changing the nature and amount of their growth regulators. This change may channel respiration in the direction of energy yielding processes.

Amaranthus chlorostachys is a widely distributed weed in Egypt. The percentage of germination of the freshly harvested seeds was found to be about 57%. Thompson and Kesar (1939) studied the effect of six sulphur compounds on the germination of lettuce seeds. They reported that thiourea is the most generally effective compound in promoting germination of lettuce seeds. Since that time, thiourea has been used by many investigators to stimulate the germination of many seeds (Delvia, 1969; Mayer and Plojloff-Mayber, 1975).

In this paper, the effect of thiourea on the germination, respiration and growth regulators of Amaranthus chlorostachys seeds will be represented.

Material and Methods

Amaranthus chlorostachys seeds were collected from the Botanical Garden of the Faculty of Education, Ain Shams University, Cairo. The seeds were either soaked in distilled water or in different concentrations of thiourea for 48 hours. After thoroughly washing the seeds, they allowed to germinate in petri-dishes lined with filter paper at 35°C. At least one hundred seeds were used in each treatment. After 72 hours incubation, the percentage of germination of the seeds was estimated.

Conventional manometric technique (Umbreit et al .,1959) was used in the respiratory studies. 0.05M potassium buffer at pH 6.0 was used as it was found to be the optimum pH for the respiration of Amaranthus chlorostachys seedlings. The Warburg water bath was at 35°C, the optimum temperature for the germination, growth and respiration of the seeds.

For the extraction and detect of the growth regulators, the seeds were soaked either in distilled water or 0.2M thiourea for 48 hours and washed thoroughly. The methods used for the extraction and assay for promoters and inhibitors were that adopted by Foda and Radwan,(1962).The seed extract was fractionated by descending paper chromatography using 80% isopropyl alcohol:20% distilled water was the running solvent.The method used to assay the gibberellins and gibberellin-like substances was the lettuce hypocotyl test(Frankland and Wareing,1960 and Crozier et al .,1970).

The air dried chromatograms were subjected to some chemical tests, where a longitudinal strip from chromatograms was sprayed with certain reagents to test for indole compounds(Kefford,1955; Powell,1959), hydroxyl groups(Swain,1953),reducing substances and amino acids(Hunt,1959; Smith,1960),Gibberellins(Jones et al .,1963; Kagawa et al .,1963) and unsaturated lactones(Swain,1953).

Results

Increasing thiourea concentrations resulted in a change in the percentage of germination of Amaranthus chlorostachys seeds(fig.1.) The percentage of germination of the seeds reached a maximum of 99% at a concentration of 0.2M thiourea. Increasing thiourea concentration above that level resulted in a decrease in the percentage of germination of the seeds to a very low value of about 20% with 0.617 concentration.

There was a gradual increase in the rate of the endogenous oxygen uptake of amaranthus chlorostachys seeds during the first four hours of soaking the seeds in water(fig.2). After that, the rate of oxygen uptake remained constant until the 52nd hour. After that time, the rate of oxygen uptake started to increase again with time. When the seeds were treated with 0.2M thiourea(fig.2), the rate of the oxygen uptake increased by increasing time. Beside the

disappearance of the first constant rate which appeared in the untreated seeds between the fourth and the fifty second hour due thiourea treatment, the rate of oxygen uptake of the treated seeds was usually higher than the untreated seeds. The rate of carbon dioxide evolution followed almost the same trend as that of oxygen uptake (fig.3), but the respiratory quotient was not affected as it was about 0.7 in the treated and untreated seeds.

The extract of the untreated seeds showed in both the coleoptile and hypocotyl tests growth inhibitor zones only (fig.4 and 5). The coleoptile tests showed seven growth inhibitor zones (R_f 0.1-0.2, 0.3-0.4, 0.4-0.5, 0.5-0.6, 0.6-0.7, 0.7-0.8 and 0.9-1.0) and the hypocotyl tests showed nine growth inhibitor zones (R_f 0.0-0.1, 0.1-0.2, 0.2-0.3, 0.3-0.4, 0.4-0.5, 0.6-0.7, 0.7-0.8, 0.8-0.9 and 0.9-1.0). All of these growth inhibitor zones gave a positive colour reaction with diazotized p-nitro alanine indicating that these growth inhibitor zones may contain unsaturated lactones. When Amaranthus chlorostachys seeds were treated with 0.2M thiourea, the growth inhibitor zones were reduced in both the coleoptile and the hypocotyl tests (fig.4 and 5). Besides, three promotor zones (R_f 0.1-0.2, 0.3-0.4, and 0.5-0.6) appeared in the coleoptile tests. The second and third zones gave a positive colour reaction with indole reagents whereas the first zone gave a positive colour reaction in the test for the hydroxyl groups. In the hypocotyl tests, the growth inhibitor zones were decreased, and one promotor zone (R_f 0.5-0.6) appeared. This zone gave a positive colour reaction in the test for hydroxyl groups.

Discussion

It was found that soaking Amaranthus chlorostachys seeds in 0.2M thiourea for 48 hours resulted in an increase in the percentage of germination of the seeds from 57% to 99% (fig.1).

The rate of respiration of Amaranthus chlorostachys seeds was found to pass through several phases during germination (fig.2 and 3). An initial rapid increase during the first four hours of soaking the seeds, a plateau when it remains constant until the 52nd hour and a second increase. This trend of the rates of oxygen uptake and carbon dioxide evolution of Amaranthus chlorostachys seeds during germination is similar to that reported by Spragg and Yean

for pea seeds. The first rise in the rate of respiration is not necessarily related to germination as such, and occurs equally in seeds which will germinate and those which will not (Mayer and Poljakoff-Mayber, 1975). The rise after the plateau occurs only in seeds which germinate and is closely associated with seedling growth. If germination is prevented in some way, the second rise in respiration is usually also prevented.

When Amaranthus chlorostachya seeds were treated with 0.2M thiourea the rate of respiration increased with time, (fig. 2&3). The plateau which appeared in the untreated seeds between the fourth and the 52nd hour of germination disappeared. Beside the disappearance of this plateau, the rate of respiration of thiourea treated seeds was higher than the untreated ones.

The fractionated extract of Amaranthus chlorostachya seeds showed growth inhibitor zones in both the coleoptile and the hypocotyl tests (fig. 4&5). When the seeds were treated with 0.2M thiourea, the growth inhibitor zones were decreased and growth promoter zones appeared.

Germination is an energy requiring process and is therefore dependent on the respiration of the seeds. Degey and May (1965) reported that thiourea is not known to have any direct effect on the respiratory enzyme system of a particular fraction similar to mitochondria isolated from lettuce seedlings. In 1960, Ustunur and Poljakoff-Mayber studied the effect of thiourea on the phosphorylating activity of lettuce seed mitochondria. They reported that thiourea stimulates the P/O ratio.

It is suggested that thiourea may act on Amaranthus chlorostachya seeds by changing the nature and amount of the growth regulators present in the seeds. This change may affect the respiratory mechanism possibly by rapidly channelling all respiration in the direction of energy yielding processes.

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References

- Crozier, A., Kuo, C.C., Durley, R.C. and Pharis, R.P.: The Biological activity of 26 gibberellins in 9 plant bioassays. *Cand. J. Bot.*, **48**:867 (1970).
- Delwin, R.M.: *Plant Physiology*. Van Nostrand Reinhold, New York (1969).
- Feda, H.A. and Radwan, S.S.A.: Straight growth test for hormones and inhibitors using coleoptiles of some Egyptian plants. *Ain-Shams, Sci. Bull.*, **8**: 381-390 (1962).
- Frankland, B. and Wareing, P.F.: Effect of gibberellia acid on hypocotyl growth of lettuce seedlings. *Nature*, **187**: 255-256 (1960).
- Hunt, G.E.: Partition chromatography and its use in the plant Science. *Bot. Rev.*, **22**: 148 (1959).
- Jones, D.P., McMillans, J. and Podley, N.: *Phytochemistry*, **2**:307 (1963). After Palag, E., 1965. Physiological effect of gibberellins. *Ann. Rev. Plant Physiol.*, **16**: 291).
- Kaga, M., Fujimura, E. and Saito, T. *Acta Biol. Pham. Tokyo*, **27**:398 (1960). (After Palag, E., 1965. Physiological effect of gibberellins. *Ann. Rev. Plant Physiol.*, **16**: 291).
- Kobayashi, K.P.: The growth substances separated from plant extracts by column chromatography. *J. Exp. Bot.*, **11**: 195 (1960).
- Kobayashi, K.P. and Poljakoff-Meyber, A.: The germination of seeds. Pergamon Press, Oxford (1967).
- Poljakoff-Meyber, A.: Qualitative analysis of substances prepared from lettuce seedlings. *J. Exp. Bot.*, **10**: 321-326 (1955).
- Powell, D.E. Jr.: Paper chromatography of the auxins. *Bot. Rev.*, **25**: 198 (1959).
- Smith, I.: *Chromatographic and Electrochemical Techniques*. William Heinemann Medical Books Ltd. London (1960).
- Spragg, S.F. and Yemm, E.W.: Respiratory mechanisms and the changes of glutathione and ascorbic acid in germinating peas. *J. Exp. Bot.*, **10**: 410-425 (1959).
- Swain, T.: The identification of coumarin and related compounds by filter paper chromatography. *Biochem. J.*, **53**:200 (1953).
- Thompson, T.S. and Kosar, W.F.: Stimulation of germination of dormant lettuce seed by sulphur compounds. *Plant Physiol.*, **14**: 567-571 (1939).
- Ullitzur, S. and Poljakoff-Meyber, A.: Oxidative phosphorylation in germinating lettuce seeds. *J. Exp. Bot.*, **14**:96-100 (1963).
- Uabreit, W.W., Burris, R.H. and Stauffer, J.F.: *Manometric Techniques*. Burgess Publ. Co. U.S.A. (1959).

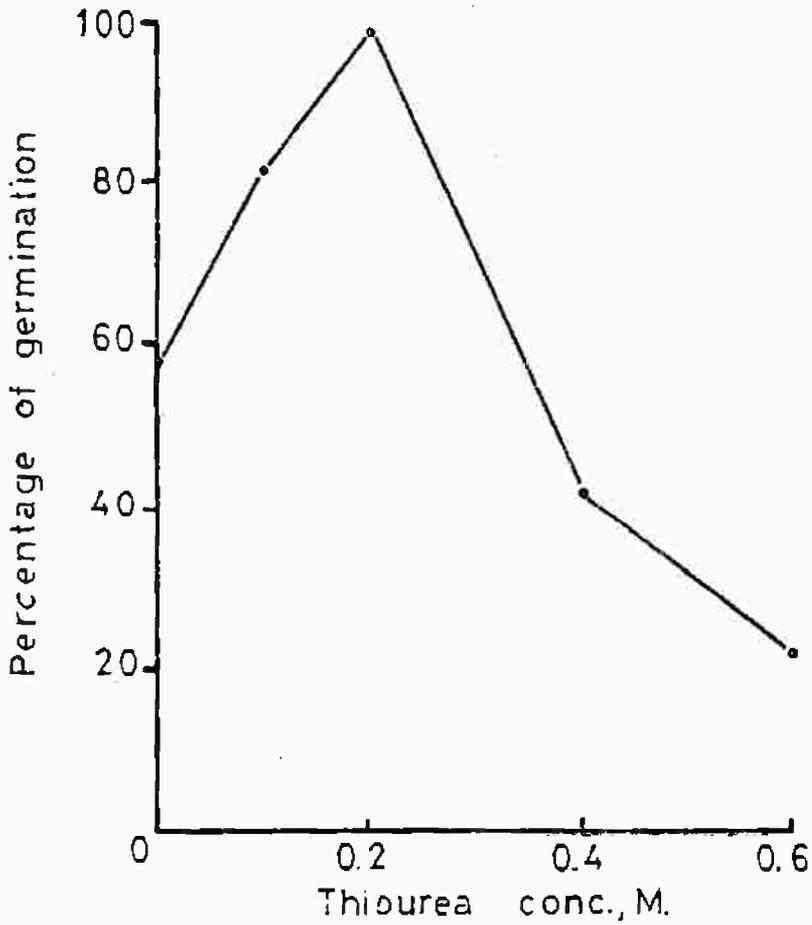


Fig.(1): Effect of thiourea on the percentage of germination of Amaranthus chlorostachys seeds

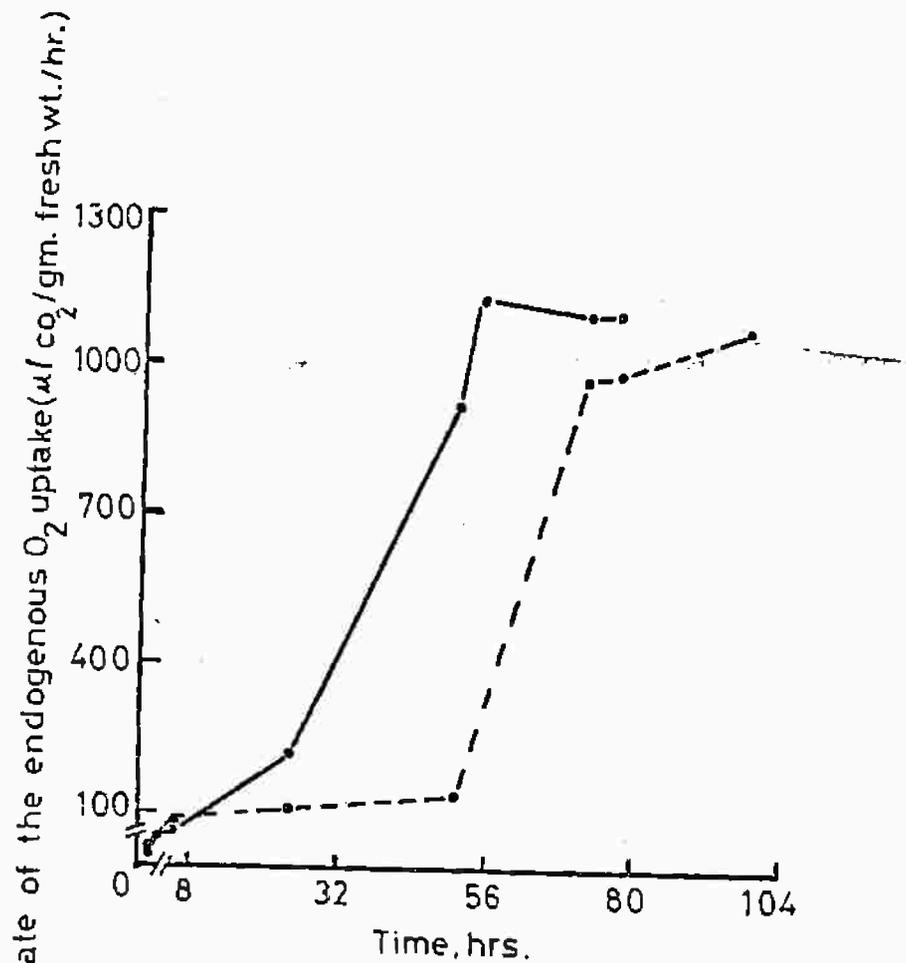


Fig. (2): Effect of thiourea on the endogenous oxygen uptake of Amaranthus chlorostachys seeds during germination.

(treated —●—, untreated - - - -●)

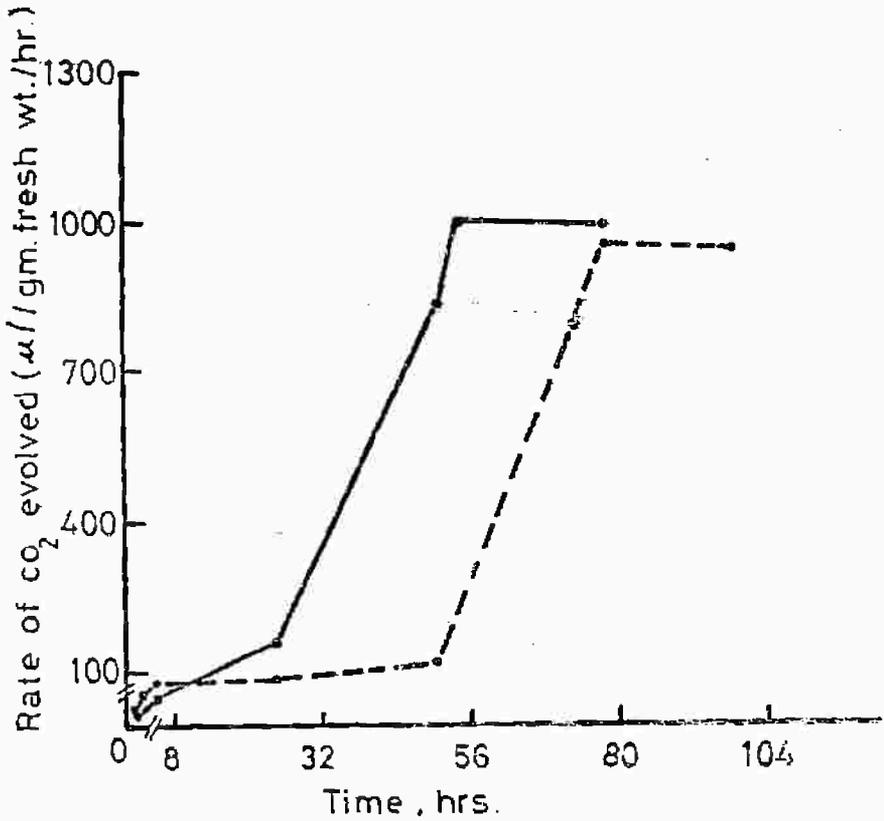


Fig.(13): CO₂ evolution of untreated and treated *Amaranthus chlorostachys* seeds with thiourea during germination.

(●—● treated, ●---● untreated.)

Change in length of coleoptile sections as % of control.

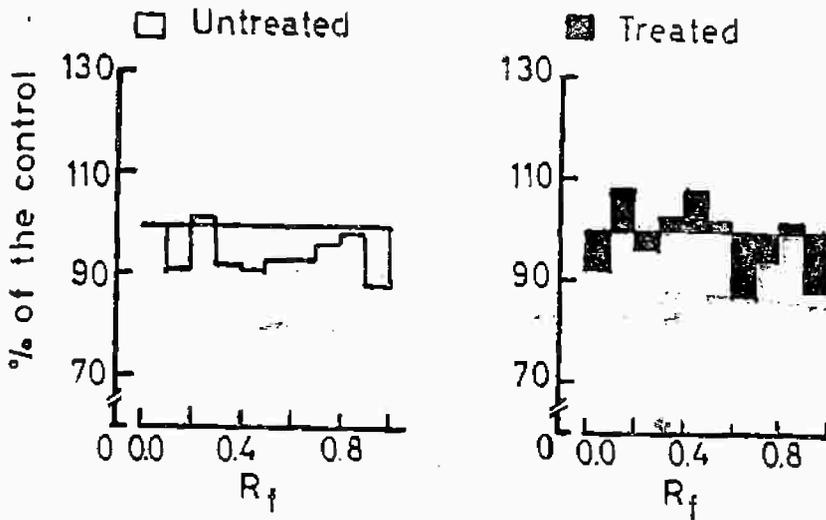


Fig.(4): Coleoptile test for fractionated extracts of thiourea treated and untreated Amaranthus chlorostachys seeds.

Change in length of hypocotyl sections as % of control.

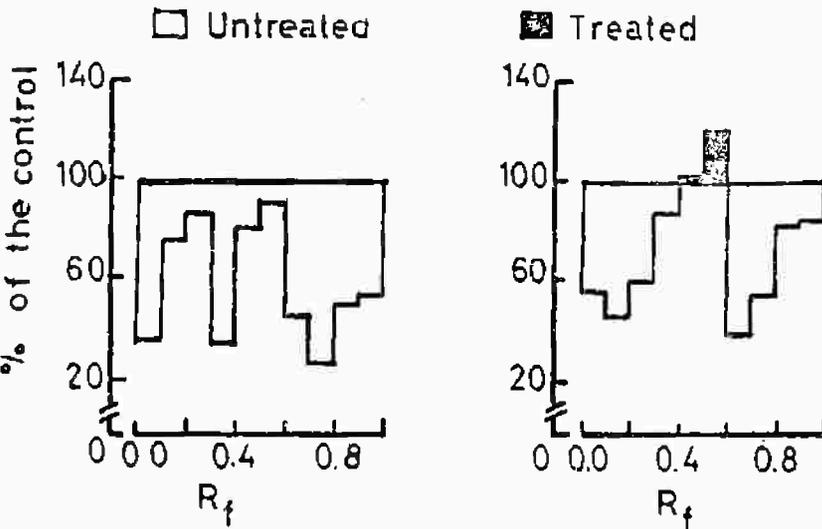


Fig.(5): Hypocotyl test for fractionated extracts of thiourea treated and untreated Amaranthus chlorostachys seeds.