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Study of Pix regulator effect on physiological responses in cotton plant

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ABSTRACT

In this study the effect of different concentrations of pix as plant growth regulators include 0 (control), 0.5 ,1, 1.5 , 2 L.ha^{-1} on soluble sugars proline, phenolic compounds content and antioxidant enzymes activity such as catalase, peroxidase and poly phenol oxidase in leaf and root of cotton plant (Gossypium hirsutum L. cv Ci-Ocra) in vegetative phase mid under pots condition were evaluated. The result showed that pix spray on cotton shoot increased amount of soluble sugars in leaf. Also pix reduced proline content in root while was not affected amount of phenolic compounds in cotton plant. Our data showed pix application in different levels had not significant effect on catalse and peroxidase activity in leaf while decreased catalase activity and increased peroxidase activity in root than control. Different treatments of pix did not change poly phenol oxidase activity in root and leaf of cotton significantly.

Key words:antioxidant enzymes, *Gossypium hirsutum L* , organic compounds, plant growth regulators

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INTRODUCTION

Plant growth regulators (PGR) are substances that affect morphological and physiological processes of plants at very low concentrations. When were produced endogenously by plants, they are referred to as plant hormones. PGR as either naturally or synthetic compounds that are applied directly to a target plant to alter its physiological processes or its structure to improve quality, increase yields, or facilitate harvesting control, undesirable vegetative growth of crop plants, enhancingfruiting bodies [9]. They like promoters, inhibitors play a key role in control mechanism of plant growth by interacting with metabolic processes such as nucleic acid and protein synthesis [10].

One of Plant growth regulators is Pix (N,N-dimethylpiperidiniumchloride), commonly referred to as Mepex, Topit, and Mepiquat Chloride and consists of 4.2 % N, N-dimethyl piperidinium chloride, a quaternary ammonia compound [18, 20].

Pix is the first plant growth regulator in cotton that have significant effect on cotton growth and yield. Gibberellins, a common plant hormone which are associated with stem elongation but have been shown to increase fruit retention in cotton. Plant growth regulators such as pix decrease cotton vegetative growth by inhibiting gibberellic acid, a common plant hormone which in turn decreases cell elongation [9]. Also it suppress vegetative growth in cotton by reducing the main stem and fruiting branch in=ternodes lengths and leaf area [12,13].

Cotton(*Gossypium hirsutum* L.) is one of the important cash crops of Iran. Cotton plays important role in the economy of the country. Vegetative growth its continues well into reproductive development. When conditions that favor vegetative growth are prevalent (e.g. excessive nitrogen or low early fruit retention), several negative effects may occur, including delayed crop maturity, flower abortion, and reduced harvest ability The cotton plant produces several natural growth regulators or plant hormones. Plant hormones work to adjust plant growth and specify energy diversion [4, 5 , 8].

Pix is used in two methods. In the first method, cotton seed is soaked into pix whereas in the second method, pix is sprayed on shoot at the beginning of flowering [24].

There are many research studies on the way pix affects cotton plant growth and development but its effects on molecular behaviors such as activities of antioxidant enzymes and osmoliths content have not been much studied. The aim of this research was to study the effect of different values of pix on soluble sugars, proline, phenolic compounds content, catalase, peroxidase, ascorbate peroxidase and poly phenol oxidase activity of cotton leaf and root.

MATERIALS AND METHODS

Planting

Eexperiments were conducted in 2009 in Gorgan city of Iran. Cotton seeds(*Gossypium hirsutum L. cv Ci-Ocra*) were placed in pots including 5 Kg of soil (Si-Clay tissue) in photoperiods 20 ± 2 ºC and 14– h light /10 –h dark and irrigated with to 350 ml water per 24 h. In 3-5 leave stage, 4 plant remained in each of pots. Then five concentrations of pix containing 0(control), 0.5 ,1, 1.5 and 2 $L.ha^{-1}$ were sprayed to shoot. Each treatment was replicated four times and arranged in a randomized complete block design.

In 6-8 leaves stage, plants were harvested and separated shoot and root and experiments were done of them.

Biochemical Analysis

Soluble sugars assay: To determine the soluble sugars, leaf and root of cotton were dried in oven at 95 °C for 24 h. They were weighed and 10 ml ethanol (70%) was added. Then the samples were placed in Petri dishes for 7 days at 4°C. Soluble sugars contents were determined by measuring the absorbance at 485 nm spectrophotometrically with Kochert [14]. Glucose standard curve was used to estimate the soluble sugars concentration (mg g^{-1} DW).

Total proteins assay: Plant samples were dried in oven at 95°C for 24h and weighed. Total proteins contents were determined at 625 nm spectrophoto-metrically using Lowry [16].method. Concentrations of total proteins were measured by bovine serum standard curve on the basis of mg g^{-1} DW.

Proline Assay: Proline content was determined by measuring the absorbance at 520 nm using Bates [1] method in leaf and root of cotton. Proline content was determined by standard curve of pure proline (µmol g^{-1} FW).

Phenolic compounds assay: For assay of phenolic compounds in cotton plant, Matta [17] method was used spectrophotometrically at 640 nm. In this method samples were boiled in 10 ml of 80% alcohol for 15 min and then centrifuged for 15 min at 3000 g. To 5 ml of this solution, 5 ml of diluted foline (1:3) and 10 ml of saturation Na_2CO_3 were added. Samples were mainted for 10 min at 25°C and then centrifuged for 15 min at 4000 g. Supernatant absorption was determined at 640 nm.Content of phenolic compounds was measured by standard curve of catechol on the basis of mg g^{-1} FW.

Enzyme Extraction

The cotton plants samples weighting about 1 g were homogenized with 4 ml extract solution containing 1.2 g tries, 2g ascorbate,3.8 g borax (Di -sodium tetra borate), 2g EDTANa2, 50g polyethylene glycol 2000 in 100 ml distilled water .The solution was placed at 4°C for 24 h and then was centrifuged for 30 min at 4000g.The clear supernatant was taken as enzyme source and used for catalase and peroxidase and polyphenol oxidase activity assay.

Catalase activity Assay

The catalase activity was assayed by Chance and Maehli [2] method with the following modification: 5 ml of assay mixture for catalase activity contained :300 µM of phosphate buffer,(pH 6.8) 100 µM of H_2O_2 and 1 ml of the twice diluted enzyme extracted. After incubation at 25°C for 1 min, the reaction was stopped by adding 10 ml of 2% (v/v) H_2SO_4 and the residual H_2O_2 was titrated against 0.01 N of $KMnO_4$ until a faint purple color persisted for at least 15 sec. One unit of catalase activity is defined as the amount of enzyme which breaks down 1 µ mol of H2O2/min under the described assay condition

Peroxidase activity assay

The peroxidase activity was determined by Koroi [15] method. 0.1 Ml of enzyme extract was added to assay mixture containing: 2ml 0.2 M acetate buffers (pH 5.0), 0.4 ml of 3% H2O2 and 0.2 ml of 0.01M benzidin solution in 50% alcohol .The activity of enzyme was determined by taking the absorbance at 530 nm . In order to protect enzyme activity, upper stages were done in ice dishes**.**

The statistical significance of the difference between parameters was evaluated by means of Duncan-test on SPSS 11.5 and for each treatment and control, four replications were selected. The results were given in the text as p, the probability values, and $p \leq 0.01$ was adopted as criterion of significance.

RESULTS

Pix effect on soluble sugars content

According to the results of this research, application of pix in concentration 2 $L.ha^{-1}$ increased soluble sugars content in cotton leaf in comparison with control. In cotton root different concentration of pix decreased soluble sugars content in comparison with control and had no significant different with other treatments(fig 1).

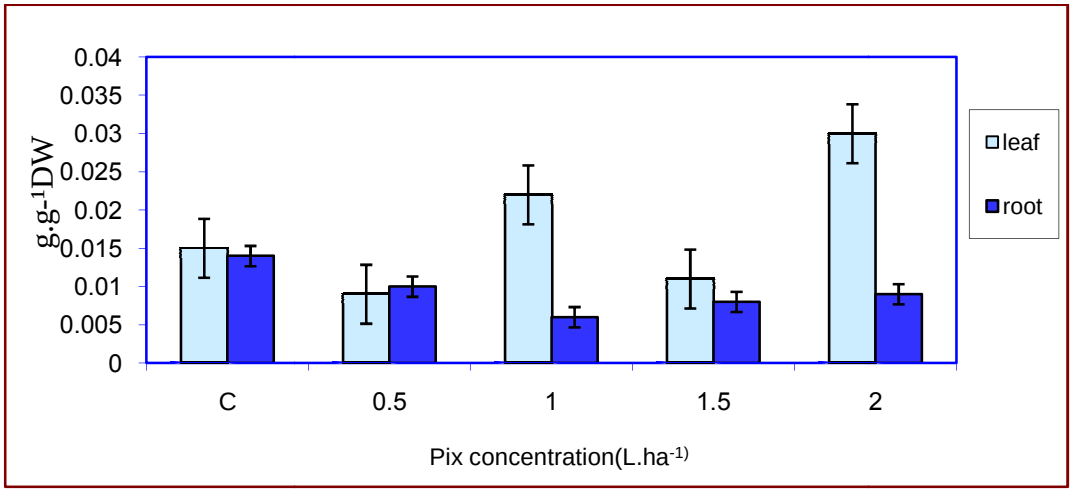

Fig 1:Effect of pix different concentrations (. = Control, 0.5, 1, 1.5 and 2 $L.ha^{-1}$) on soluble sugars in leaf and root of cotton .(X±SE)

Pix effect on proline content

As it was seen in fig 2 spraying of pix in different concentrations cause decrease proline content in leaf cotton in comparison with control. In root also pix application decreased proline content significantly which this decreasing in treatments of 1.5and 2 L. ha^{-1} of pix were considerable.

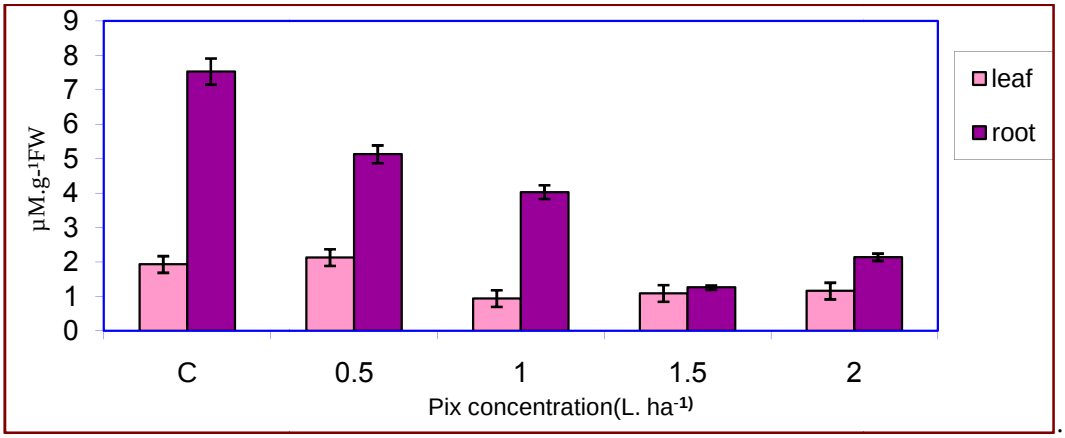

Fig 2:Effect of pix different concentrations (. = Control, 0.5, 1, 1.5 and 2 $L.ha^{-1}$) on proline content in leaf and root of cotton . (X±SE)

Pix effect on phenolic compounds content

The effect of different amounts of pix on phenolic compounds content in leaf and root of cotton fig 3 was shown. The results of this assay indicated that pix different concentrations had not significant effect on amounts of phenolic compounds in cotton .

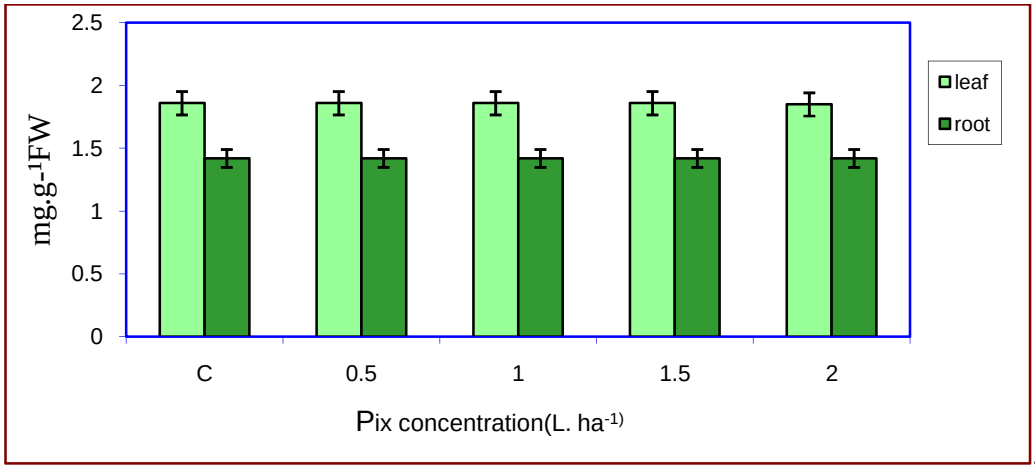

Fig 3::Effect of pix different concentrations (. = Contro, 0.5, 1, 1.5 and 2 L.ha^{-1}) on phenolic compounds content in leaf and root of cotton . (X±SE)

Pix effect on antioxidant enzymes activity

Catalase activity

According to the results of this research, application of pix different concentrations did not have any significant effect on catalase activity in leaf cotton while spraying of pix in the highest concentrations (2L. ha^{-1}) decreased activity this enzyme in comparison with control and other treatments significantly (fig 4).

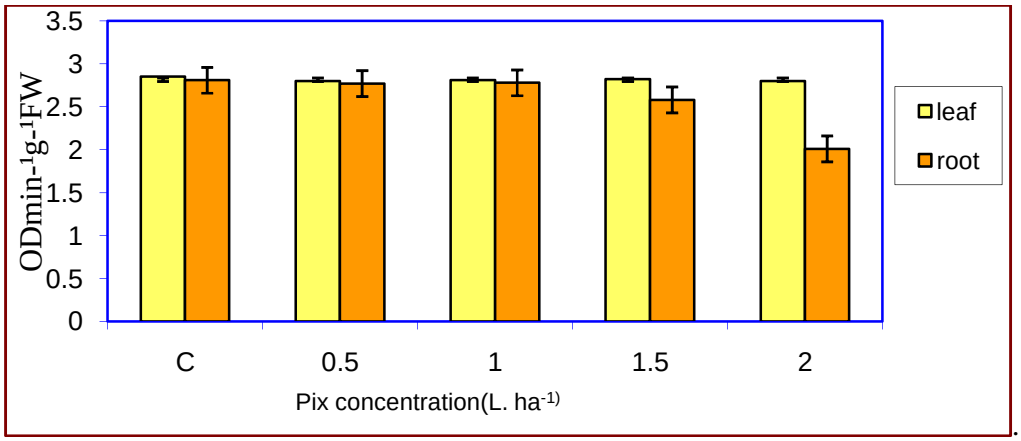

Fig 4::Effect of pix different concentrations (. = Control, 0.5, 1, 1.5 and 2 L.ha^{-1}) on catalase activity in leaf and root of cotton . (X±SE)

Peroxidase activity

The results showed that pix different concentrations did not change peroxidase activity in cotton leaf while in root increased enzyme activity on concentration 1.5 and 2 L. ha^{-1} in comparison other treatment and control (fig5).

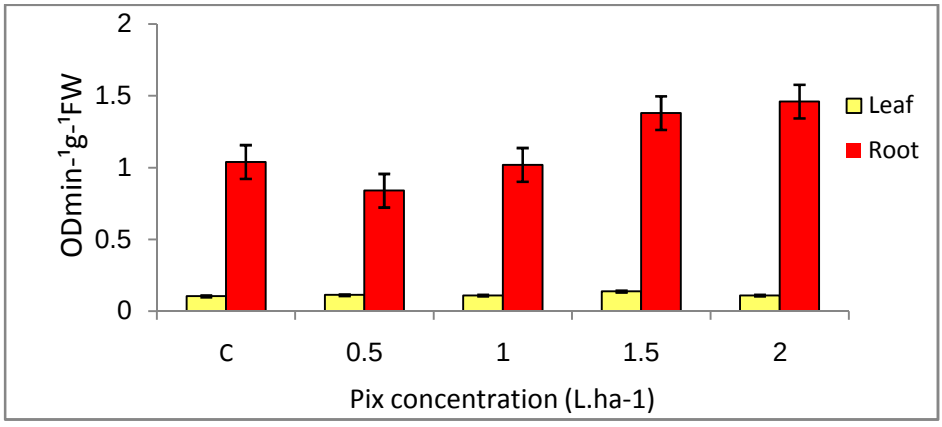

Fig 5:Effect of pix different concentrations (. = Control, 0.5, 1, 1.5 and 2 L.ha^{-1}) on perxidase activity in leaf of cotton. (X±SE)

Poly phenol oxidase

As it was seen in fig 6 spraying of pix in different concentrations had not significant effect on poly phenol oxidase activity in leaf and root of cotton (fig 6).

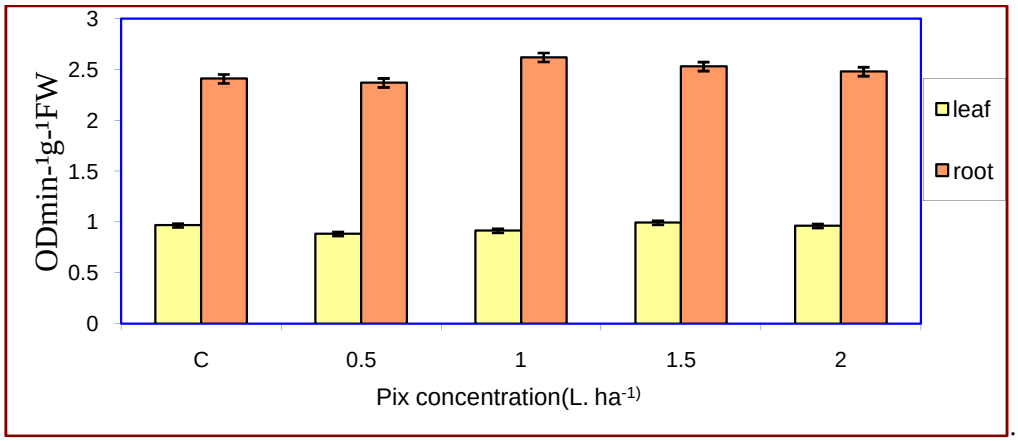

Fig 6::Effect of pix different concentrations (. = Control, 0.5, 1, 1.5 and 2 L.ha^{-1}) on polyphenol oxidase activity in leaf and root of cotton . (X±SE)

DISCUSSION

Our results indicated that pix application in concentration 2 L. ha-1 increased soluble sugars in leaf and decreased them in root in comparison with control (fig1). Plant growth regulators such as pix decrease cotton vegetative growth by inhibiting gibberellic acid [18]. Gibberellic acid is a natural plant hormone that increases the α- amylase activity in seeds and plants. This enzyme breaks down starch and converts it into glucose [11]. This seems to be related to lack of amylase activity in the presence of 2 L. ha-1value of pix.The effect of pix on soluble carbohydrate raising in cotton also was shown by Gopalakrishnan, et al [5] and Muhammad et al[18].

The research showed pix plus increase the levels of the sugar alcohols (polyols) which is believed to be related to the improved partitioning of dry matter into cotton bolls [6, 25].It was reported leaf carbohydrates represent the primary metabolic carbohydrate pools for cotton thus understanding of their dynamics during cotton growth and boll development is important. It was said plant growth regulators (PGR) can influence on carbohydrate translocation out of the cotton leaf .The use of14carbon-labeling techniques confirmed the influence of PGRs as pix on carbohydrate translocation out of the leaf. The enhance translocation of carbohydrates out of the leaf was associated with an increase in leaf photosynthesis and a yield advantage. Photosynthesis is often improved when PGRs are used.The carbohydrate balance of reproductive tissues strongly influences reproductive success in cotton [26,23]

Since leave samples were taken in the early phase of reproductive and boll formation had not yet, so carbohydrate did not move from the leaves of the boll. At this time, pix application increased photosynthesis rate and subsequent carbohydrate content also raised.

It seems pix to move carbohydrates from the roots to the leaves on the carbohydrates rise also was effective.

Our data analysis showed that spraying of pix in different concentrations cause decrease proline content in leaf and root of cotton in comparison with control that this decreasing in root was considerable (fig 2).It was reported pix application enhanced protein content in plant [22].

According to report of Mundree et al [19] proline reduction in cotton leaf and root in treatment with pix can related to proline oxidase activity which catalysis convert proline to glutamine that was used it in biosynthesis other amino acids and proteins.

The results also showed that amount of phenolic compounds was not affected by different concentrations of pix because the difference between control and treatments was not significant (fig3). It seems phenolic compounds in cotton plant is not sensitive to the Pix values in this experiment and or pix have not interaction to enzyme responsible biosynthesis and destruction these compounds. Hampton and Oosterhuis[7] suggested that phenolic compounds modify growth and development of cotton fruit during stress, and indicated the potential for use of

phenolic acids as growth regulators in cotton. Since in this research cotton plants were not under stress condition, it seems change in phenolic compounds content in plant was not essential.

Figures 4-6 shows effect of pix treatments on antioxidant enzymes in leaf and root of cotton. According to our results different concentrations of pix had not significant effect on catalase and peroxidase activity in leaf. It seems that activity these enzyme in leaf is not sensitive to the pix values in this experiment. On the other hand the highest of pix concentration (2 L. ha-1) decreased catalase activity and increased peroxidase activity in cotton root. It was reported exogenous application of gibberellic acid reduced the peroxidase activity in rice seedling [11]. Gibberellic acid stops peroxidase production in spinach plant. Peroxidase limits the growth by hardening the cell wall. Gibberellic acid reduces the strength and hardness through the inhibition of peroxidase production. Peroxidase reversing the balance between cell wall phenolic polymers, decreases cell wall elasticity [21]. Plant growth regulators such as pix inhibit gibberellic acid synthesis [18], thus peroxidase activity increase in presence of pix.

Our results showed that treatment with pix did not change poly phenol oxidase activity in cotton leaf and root (fig 6). It is reported that substrates of these enzymes are phenolic compounds [3]. Since in this study phenolic compounds content did not affect pix different treatment, no change of polyphenol oxidase activity in leaf and root was predict.

CONCLUSION

PGRs such as pix allow for manipulation of physiological processes in plant growth and development for more efficient crop management and increased yields. Our research showed that the use of pix special in concentration 2 $L.ha^{-1}$ increased soluble sugars in leaf cotton and decreased proline content, catalase activity in root cotton.Also pix had not significant effect on phenolic compounds, catalas and peroxidase activity in leaf while its effect on peroxidase activity increasing was considerable in root. Continued research at both applied and basic levels will elucidate the specific effects and mode of action of PGRs and thereby aid in improving their performance and consistency.

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