Effect of pix on germination, growth, carbohydrates and antioxidant enzymes in cotton seed

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Abstract

Pix is a plant growth regulators that interferes with other hormones and affect the germination and growth of cotton. In this study the effect of different concentrations of pix, namely, 0 (control), 10, 20, 30 and 40 ppm on germination, radicle dry weight, radicle length, insoluble and soluble sugars and antioxidant enzymes activity (catalase, peroxidase and poly phenol oxidase) in cotton seedling (*Gossypium hirsutum* L. cv Ci-Ocra*)* in Petri dishes were examined. The results showed that germination percentage and radicle growth increased in concentration of 20 ppm of pix, also radicle dry weight reduced at concentrations 10 and 40 ppm. In addition, analysis of data revealed that different concentrations of pix did not affect catalase enzyme activity while it increased peroxidase and poly phenol oxidase activity as compared to the control.

*Keywords***:** cotton; pix; germination; carbohydrate; antioxidant enzyme

Niakan, M., A. Habibi and **M. Ghorbanli.** 2011. 'Effect of pix on germination, growth, carbohydrates and antioxidant enzymes in cotton seed'. *Iranian Journal of Plant Physiology* 1 (4), 301 – 307**.**

Introduction

Cotton (*Gossypium hirsutum)* is a subtropical, perennial plant with an indeterminate growth habit. Its vegetative growth continues well into reproductive development. When conditions favoring 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. Cotton plant produces several natural growth regulators or plant hormones (Ghourab et al., 2000; Gopalakrishnan et al., 2004; Harish et al., 2003). Plant growth regulators are compounds that affect cotton growth and development. One of these compounds is pix (N, N-dimethyl piperidinium chloride: pix), commonly referred to as Mepex, Topit, and Mepichlor and consisting 4.2% N, N-dimethyl piperidinium chloride, a quaternary ammonia compound (Muhammad et al., 2007; Najma et al., 2000).

Plant growth regulators such as pix decrease cotton vegetative growth by inhibiting gibberellic acid, a common plant hormone which in turn decreases cell elongation (Havargi, 2007). Synthetic plant growth regulators also suppress vegetative growth in cotton by reducing the main

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Received: July, 2011
Accepted: September 2011

stem and fruiting branch internodes length and leaf area (Jonathan and Alexander, 2006; Joseph and Johnson, 2006). Reduced leaf area is caused by suppressed cell enlargement. Lower leaf area also improves pesticide distribution into the lower canopy. More importantly, lower Leaf Area Index allows more light to penetrate the lower canopy for boll development as most photosynthetic energy for boll development originates from adjacent or subtending leaves. Thus, boll development in a shaded environment may result in reduced maturity, yield, and quality (Prasad and Ram, 2000; Ram, 2001).

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 (Thandapani, and Subharayalu, 2000).

There are many research studies on the way pix affects cotton plant growth and development but its effects on germination and molecular behaviors such as activities of antioxidant enzymes have not been much studied. Various plant growth regulators have also been used in pre-treating the seeds to induce drought resistance. Plant growth regulators have been viewed as chemical messengers regulating the normal progression of developmental changes as well as responses to environmental signals (Muhammad et al., 2007). Germination and seedling development is one of the important factors in determining final yield (Derrick, 2000). Seed treatment by pix before planting can be a successful tool in cotton production. Interfering with hormones, pix influences germination and growth of cotton seedling (Thandapani, and Subharayalu, 2000). The aim of this research was to study the effect of different values of pix on germination, catalase, peroxidase, ascorbate peroxidase and poly phenol oxidase activity of cotton seedlings.

Materials and Methods

Cotton seeds (*Gossypium hirsutum* L. cv Ci-Ocra*)* were sterilized with Carboxyl Tiraman (vitax) for a rate of 4 g per kg seeds. Then 15 seeds were placed in Petri dishes and separately soaked in different solutions of pix including 0 (control) 10, 20, 30 and 40 ppm and lined with Whatman Filter Paper (No2). Petri dishes were then kept in germinator (25 ± 1 °C and 60% humidity) for 7 days. Seed germination was recorded at 24, 36 and 48 h. Seeds with root length ≥ 2 mm were considered as germinated seeds. Germination percentage was calculated with *GP=100n/N* where *GP* was germination percentage, *n* represented the number of germinated seeds and *N* was total number of seeds (Weston, 2003). Root length was also measured with a ruler (mm) on the seventh day.

Enzyme extraction

The cotton seedling samples weighting about 1 g were homogenized with 4 ml extract solution containing 1.2 g Tris, 2 g ascorbate, 3.8 g borax (Di -sodium tetra borate), 2 g EDTANa2, 50 g 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 4000 g. The clear supernatant was taken as enzyme source and used for catalase and peroxidase activity assay.

Catalase activity assay

The catalase activity was assayed by Chance and Maehli (1995) 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 was 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 M of H_2O_2/min under the described assay condition.

Peroxidase activity assay

The peroxidase activity was determined by Koroi (1989) method. Accordingly, 0.1 Ml of enzyme extract was added to assay mixture containing 2 ml 0.2 M acetate buffer (pH 5.0), 0.4 ml of 3% H_2O_2 and 0.2 ml of 0.01 M benzidin solution in 50% alcohol. The enzyme activity was

determined by taking the absorbance at 530 nm. In order to protect enzyme activity, upper stages were done in ice dishes.

Ascorbate peroxidase activity assay

In order to determine ascorbate peroxidase activity, 2 ml phosphate buffer 0.05 M (pH 6.5) was mixed with 0.2 ml H_2O_2 3 %, 0.2 ml ascorbate 50 µM in ice dishes and 0.1 ml enzyme extract was added to them. The enzyme activity was determined by taking the absorbance at 265 nm according to Arrigonia (1994).

Poly phenol activity assay

The activity of poly phenol oxidase was assayed by Manoranjan and Mishram method (1976) with the following specifications: 2 ml of assay mixture for catalase activity was prepared including 1.5 ml of 0.2 M phosphate buffer (pH 7.6), 0.4 ml pyrogallol 0.02 M and 0.1 ml enzyme extract. This mixture was incubated at 28 °C for 3 min. The enzyme activity was spectrophotometrically determined by measuring absorbance at 430 nm.

Soluble sugars assay

In order to determine soluble sugars, seventh day cotton seedlings were dried in 110 °C for 48 h. They were then weighed and added to 10 ml ethanol 70% and were placed in poly ethylene dishes for 7 days at 4 °C. Soluble sugars contents were spectrophotometrically determined by taking the absorbance at 485 nm through the Kochert (1978) method. Glucose standard curve was used to determine soluble sugars concentration.

Statistical analysis

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

Results

Pix effect on germination

Fig. I shows germination percentage of cotton seedling during 48 h. After 48 h the highest germination rate was seen in concentration 20 ppm of pix. Germination was the least after 48 h in control and at concentration of 30 ppm.

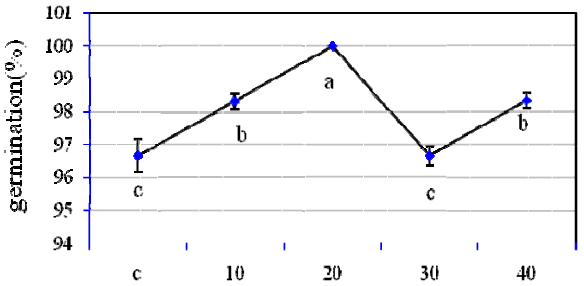

Fig. I. Effect of different pix concentrations (10, 20, 30 and 40 ppm) on cotton seed germination after 48 h. The different character = significantly different (P≤0.01).

Pix effect on radicle length and dry weight

The results showed that radicle length of cotton seedling in 20 ppm concentration of pix increased and this increase was significant in comparison with other treatments (Fig. II). Most dry weight of cotton seedling was observed in control and in concentrations 20 and 30 ppm of pix and between these treatments and concentrations of 10 and 40 ppm differences were significant (Fig. III).

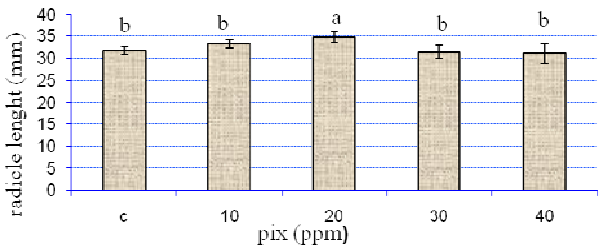

Fig. II. Effect of different pix concentrations (10, 20, 30 and 40 ppm) on radicle length of seven day old cotton seedlings. The different character = significantly different (P≤0.01).

Pix effect on soluble and insoluble sugars

The results showed that soluble sugars content in 10 ppm of pix concentration

decreased. This decrease was significant in comparison with other treatments (Fig. IV).

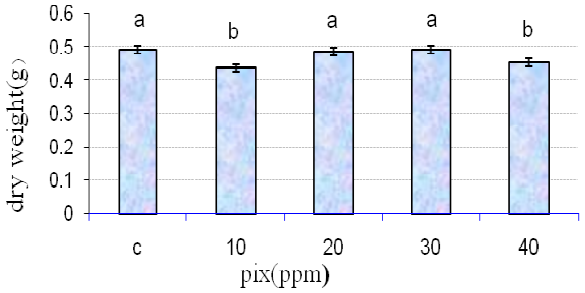

Fig. III. Effect of different pix concentrations (10, 20, 30 and 40 ppm) on dry weight of seven day old cotton seedlings. The different character = significantly different (P≤0.01).

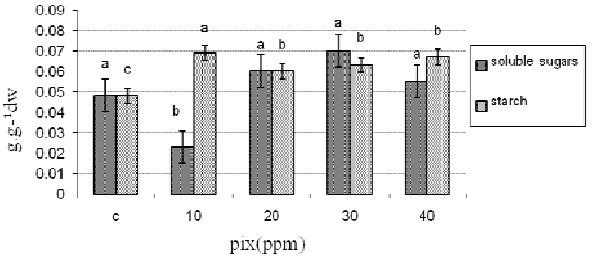

Fig. IV. Effect of different pix concentrations (10, 20, 30 and 40 ppm) on soluble and insoluble sugars of seven day old cotton seedlings. The different character = significantly different (P≤0.01).

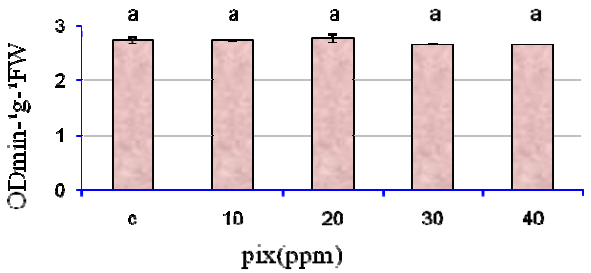

Fig. V. Effect of different Pix concentrations (10, 20, 30 and 40 ppm) on catalase activity of seven days old cotton seedlings. The different character = significantly different (P≤0.01).

Pix effect on antioxidant enzyme activity

According to the results of this research, application of different pix concentrations did not have any significant effect on catalase activity in cotton seedling (Fig. V). The effect of different amounts of pix on peroxidase activity in cotton seedling is shown in Fig. VI. Pix treatment in concentration 30 ppm causes an increase in peroxidase activity in comparison with control. As Fig. VII shows, application of pix in concentration of 40 ppm significantly increased poly phenol oxidase activity in comparison with other treatments.

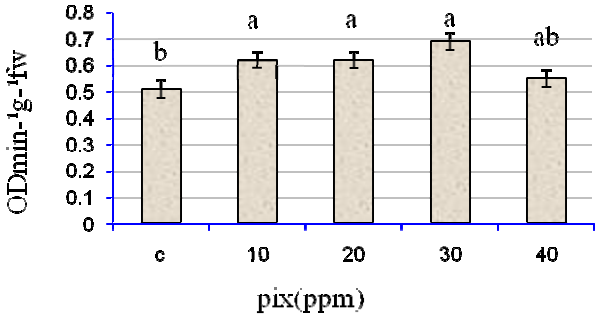

Fig. VI. Effect of different Pix concentrations (10, 20, 30 and 40 ppm) on peroxidase activity of seven day old cotton seedlings. The different character = significantly different (P≤0.01).

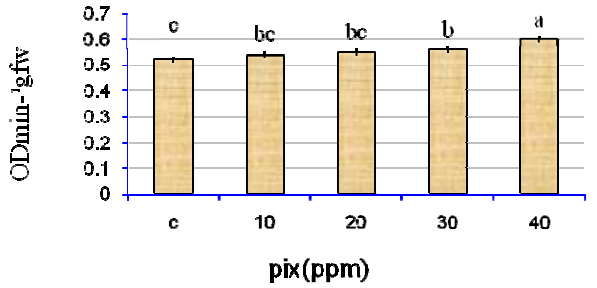

Fig. VII. Effect of different Pix concentrations (10, 20, 30 and 40 ppm) on polyphenol oxidase activity of seven days old cotton seedlings. The different character = significantly different (P≤0.01).

Discussion

The findings showed that germination percentage and radicle growth increased in concentration 20 ppm of pix (Figs. I and II). Researchers have shown that plants respond to certain concentrations of hormones (Sativrkaur et al., 2000; Sawan et al., 2000; Singh and Kakryalya, 2000; Dodds et al., 2010). Since the plant growth regulators such as pix act like hormones, in this study pix appears to have stimulating effects on germination and root growth only at concentration 20 ppm. It is reported that through stimulating activity of enzymes such as cellulase and pectinase, pix affects the cell wall increasing relaxation and flexibility of seed cell wall followed by an increase in the germination rate and root growth (Vijayakumari and Janardhanan, 2003).

Also our data analysis showed that dry weight of cotton seedlings decreased only in 10

ppm concentration of pix and this reduction was significant in comparison with other treatments (Fig. III). Results of this study for concentration 10 ppm of pix are consistent with an earlier study reported by Zhao and Oosterhuis (2000) who observed that the use of pix reduced seedling dry weight.

Figure (IV) shows changes in soluble sugars and starch content in cotton seedling. According to our results the amount of starch in all treatments decreased compared to the control. The highest of starch content and the lowest of soluble sugars in cotton seedling were seen in concentration 10 ppm pix (Fig. IV). Plant growth regulators such as pix decrease cotton vegetative growth by inhibiting gibberellic acid (Muhammad et al., 2007). 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 (Isabel and Jennifer, 2001). In this experiment, all treatments containing concentrations of 10, 20, 30 and 40 increased the amount of starch as compared to the control. This seems to be related to lack of amylase activity in the presence of different values of pix. These results are consistent with results of other researchers (Gopalakrishnan, et al., 2004; Muhammad, et al., 2007).

The results also showed that catalase enzyme activity was not affected by different concentrations of pix as the difference between control and treatments was not significant (Fig. V). It seems that catalase in cotton seedling is not sensitive to the pix values in this experiment. On the other hand, cotton seed treatments with different concentrations of pix increased peroxidase activity as compared with the control (Fig. VI). Exogenous application of gibberellic acid reduced the peroxidase activity in rice seedling (Isabel and Jennifer, 2001). 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 (Potter and Fry, 2000). In the present study it was observed that the rate of peroxidase activity in all pix treatments was higher than the control. This is consistent with the study reported by Nagashima et al. (2005) which announced that pix prevents the synthesis of gibberellic acid and subsequently increases peroxidase activity.

Our results showed that treatment with pix increased poly phenol oxidase activity and this increase was significant at concentration 40 ppm (Fig. VII). It was reported when aleuron layers of barley were treated with gibberellic acid, the content of phenolic compounds was reduced (Gubler and Ashford, 2004). Poly phenol oxidase is available to significant amounts in most plant tissues and the ROS are converted to mono and dihydroxy phenolic compounds. Substrates of these enzymes are phenolic compounds (Coetzer et al., 2001). In this study, the activity level of poly phenol oxidase in all concentrations of pix was increased and this increase was significant compared to the control. Since phenolic compounds are the substrate of poly phenol oxidase enzyme, pix appears to increase the phenolic compounds and subsequently, enzyme activity. Another possibility is that pix directly affects synthesis of the enzyme and this increases its concentration and activity.

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