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# EFFECT OF SODIUM FLUORIDE AND SOIL AMENDMENTS ON THE ACTIVITY OF POLYPHENOL OXIDASE, PEROXIDASE AND CATALASE IN *RAPHANUS SATIVUS* VAR. ARK NISHANT

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Effect of sodium fluoride on the activity of peroxidase, polyphenol oxidase and catalase was studied in radish leaves with mineral nutrient amendments. Sodium fluoride inhibited polyphenol oxidase and peroxidase, where as the activity of catalase was increased. Calcium had better ameliorative effect on the enzymes studied whereas magnesium and phosphorus showed less response.

**Keywords:** Mineral nutrients; Oxidative-enzymes; Sodium fluoride.

## Introduction

Fluoride affects a number of enzymes in plants. Some enzymes are inhibited whereas some others are activated[1]. Several oxidative enzymes are affected by fluoride[2]. Sensitivity of several enzymes to fluoride is influenced by many factors[3]. Present experiment is set up to find out the effect of soil applied sodium fluoride with mineral nutrient amendments on radish plants (*Raphanus sativus* L. var. Arkanishant).

## Material and Methods

Radish plants were grown in clay pot (30 cm & 30 cm) containing known amount of red soil. Initially for the first four weeks all the plants were supplied with one liter of deinoised glass-distilled water per pot per day until harvest except on the day when plants were treated with nutrients and fluoride solution. Thirty day-old plants were treated with one liter of Hoagland's nutrient solution (Basal medium) along with sodium fluoride (1.5mM) once a week for four weeks. After 60 days, leaves were used for extraction and estimation of enzymes.

Treatments: 1) BM-F,2) BM+F,3) BM+Ca+F,4) BM-Ca+F,5) BM+Mg+F, 6) BM-Mg+F, 7) BM+P+F and 8) BM-P+F. BM = Basal Hoaglands' nutrient medium (Calcium = $5 \times 10^{-3}$ M, Magnesium = $2 \times 10^{-3}$ M, Phosphorous $1 \times 10^{-4}$ M); +F=Sodium fluoride 1.5mM; -F=without fluoride; +Ca, +Mg, and +P indicates Calcium $10 \times 10^{-3}$M, Magnesium $4 \times 10^{-3}$ M and phosphorus $2 \times 10^{-4}$ M content in the basal medium. -Ca, -Mg and -P refers to their deletion from basal medium.

To extract crude enzymes, one gramme of fresh leaf material was placed in pre-cooled mortar and pestle and ground with 10ml of cold Tris-HCl buffer 0.05M pH 7.0 the extract was passed through cheese cloth to and centrifuged at 1000xg to remove cellular debris. The supernatant was centrifuged again at 3500xg for 20 minutes the resultant supernatant was used as the crude enzyme source for the estimation of catalase, peroxidase and polyphenol oxidase.

Catalase activity was estimated as per the method of Barber[4] . The reaction mixture contained one ml of enzyme, 2ml of $H_2O_2$ (0.005M) and 3ml of Tris-HCl buffer pH 7.0. The reaction was stopped by adding 10ml of 2.5 N $H_2SO_4$ . After one minute of incubation at 20° C, the residual $H_2O_2$ was titrated with 0.011N $KMnO_4$ . A blank was prepared by adding the extract to an acidified solution of reaction mixture at zero time. Catalase activity was expressed as mg $H_2O_2$ oxidised/g fresh weight/ minute.

Peroxidase activity was estimated as per method of Kar and Mishra[5] . The reaction mixture containing 2ml of Tris-HCl buffer 0.1M pH 7.0, lml of pyrogallol 0.01M,lml of $H_2O_2$ 0.005M was prepared. The reaction was started by adding lml of enzyme solution and the mixture was incubated at 25° C for 5 minutes the reaction was stopped by adding lml of 2.5N $H_2SO_4$. The amount of pyrogallin formed was estimated by measuring the absorbance at 425nm in shimadzu UV-VIS-spectrophotometer. The enzyme activity was expressed as change in absorbance units.

Polyphenol oxidase activity was estimated as per the method of Kar and Mishra[5] . The reaction mixture containing the following was prepared 2ml of Tris buffer 0.1M at pH 7.0, lml of pyrogallol 0.01M and lml of enzyme solution. The reaction was started by adding the enzyme solution and incubating the mixture for 5 minutes. The reaction was stopped by adding lml of 2.5N $H_2SO_4$ . Absrobancy was measured at 425 nm.

**Results and Discussion**

Sodium fluoride inhibited polyphenol oxidase activity in all the treatments. Addition of calcium ($10x10^{-3}$ M) reduced the inhibition of the enzyme of fluroide as compared to its deletion. Magnesium and phosphorus amendments did not have any significant effect on the enzyme activity (Fig.1). Peroxidase activity was inhibited in fluoride treated plants except in treatment with calcium ($5x10^{-3}$ M), magnesium ($2x10^{-3}$ M) and phosphorus ($1x10^{-4}$ M).. Calcium $10x10^{-3}$M), reduced the inhibitory effect of sodium fluoride on the peroxidase (Fig.2). Catalase activity increased with fluoride in all the treatments. Nutrient amendments with magnesium and phosphorus did not show much effect (Fig.3).

The decrease in the activities of polyphenol oxidase and peroxidase by fluoride in radish leaves can be attributed to the sensitivities of these enzymes to sodium fluoride. In both the cases calcium countered the inhibitory effect of fluoride.

The activities of polyphenol oxidase, peroxidase were also studied by Lee *et al*[2] . in fluoride fumigated soybean leaves besides *in vitro* effect of fluoride on these enzymes. They also found that the activities of peroxidase and polyphenol oxidase was suppressed throughout the fumigation period. Increase in catalase activity in radish leaves due to fluoride may be taken as a response to toxicity of fluoride as a stress factor. Similar observation were made in fluroide fumigated leaves and wounded leaf tissues by Lee *et al*[2]. Fluoride caused increase in

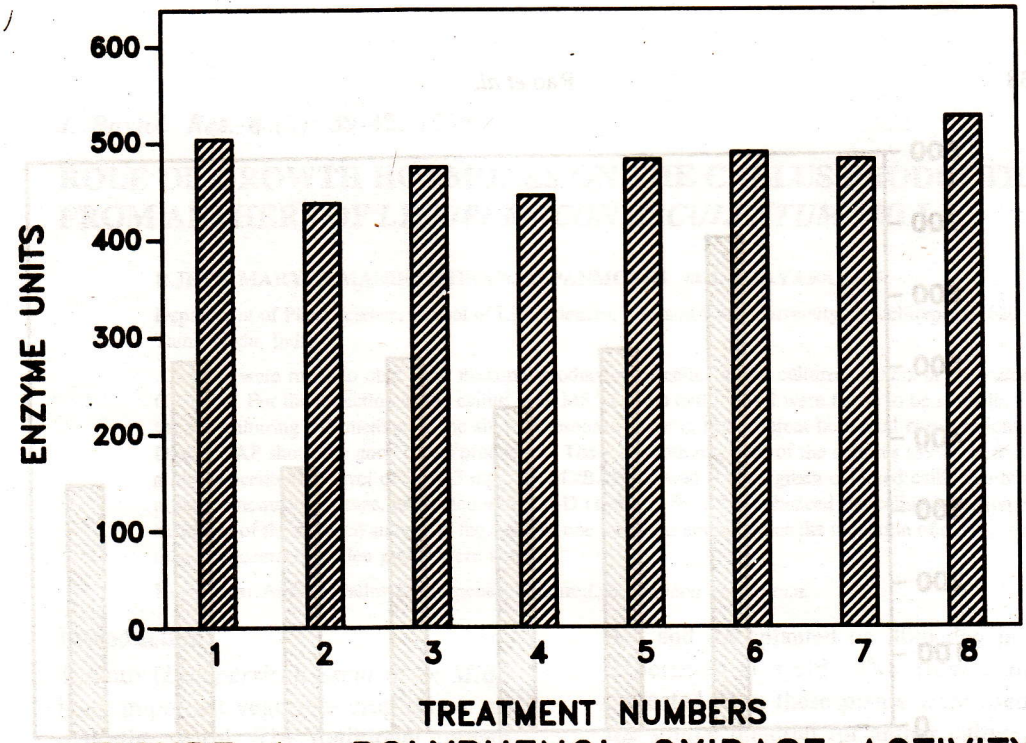


FIGURE 1 POLYPHENOL OXIDASE ACTIVITY

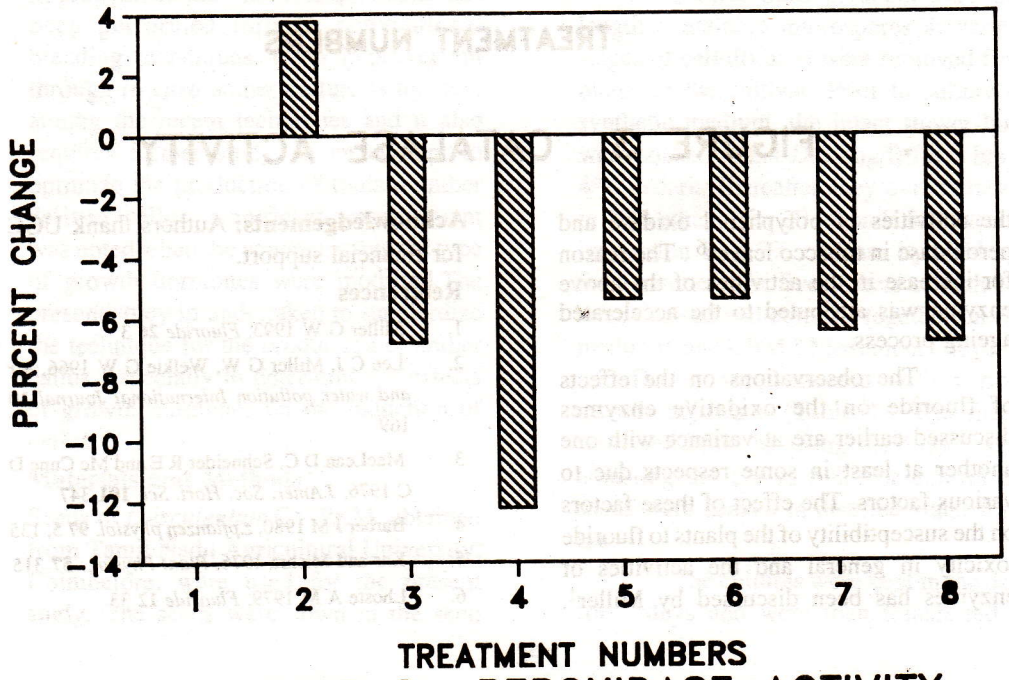


FIGURE 2 PEROXIDASE ACTIVITY

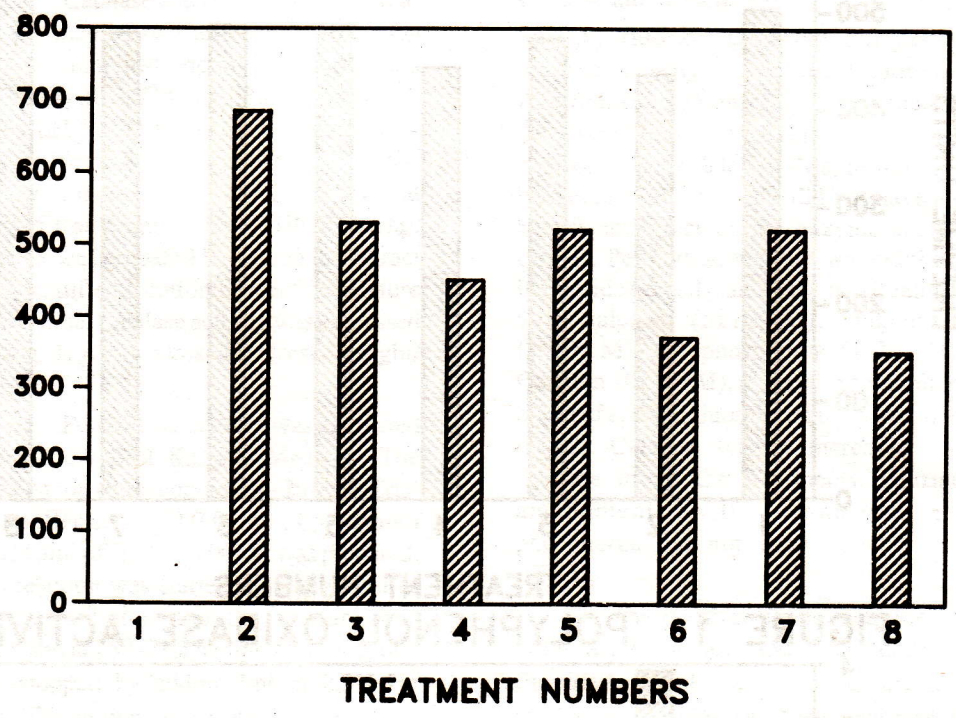


FIGURE 3 CATALASE ACTIVITY

the activities of polyphenol oxidase and peroxidase in tobacco leaves[6] . The reason for increase in the activities of the above enzyme was attributed to the accelerated ageing process.

The observations on the effects of fluoride on the oxidative enzymes discussed earlier are at variance with one another at least in some respects due to various factors. The effect of these factors on the susceptibility of the plants to fluoride toxicity in general and the activities of enzymes has been discussed by Miller[1].

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