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Role of endosulfan in mediating stress responses in *Sorghum bicolor* (L.) Moench

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Abstract: *Present study revealed the importance of endosulfan in mediating stress responses in Sorghum bicolor L. Moench (variety JP-1-1). The seeds treated with different concentrations (0.2, 0.4 and 0.6%) of endosulfan showed a significant decrease in percent germination over control. As the concentration increased, the shoot length, root length and biomass decreased. The amount of chlorophyll-a and protein decreased gradually with the increase in endosulfan concentration, whereas phenol and proline contents increased from 1.08 to 1.57 mg g^{-1} and 0.18 to 0.98 mg g^{-1}, respectively. Chlorophyll-b decreased in 0.2% (0.97 mg g^{-1}) as compared to control and revealed a gradual increase in 0.4% (1.11 mg g^{-1}) and 0.6 % (1.13 mg g^{-1}). Endosulfan treatment suppressed the catalase and protease activity, but significantly increased the level of peroxidase, polyphenol oxidase, SOD and amylase enzymes. Lower dose (0.2%) of endosulfan stimulated the activity of amylases.*

Key words: *Endosulfan, Sorghum bicolor, Biochemical constituents, Oxidative stress*
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Introduction

Large-scale application of pesticides, insecticides and other chemicals have imbalanced the natural process of both terrestrial and aquatic ecosystem. The organochlorinated pesticides pose a constant threat to non-target organisms and are known to alter the behavioral changes, growth, nutritional value, cellular and physiological properties (Mukhopadhya and Dehadrai, 1980; Sarnaik *et al.,* 2006). Insecticides affect the normal functions of specific cell and tissues of plants and make their survival difficult (Bhide, 1986). It was reported that administration of insecticides cause histopathological changes in plants (Singh, 1980, 1990). A toxic effect of many fungicides, herbicides and insecticides on germination and seedling growth was reported by many workers (Huffman and Jacoby, 1984; Jaiprakash and Singh, 1984; Gupta and Yadav, 1986). Endosulfan is a widely used insecticide on cereals, pulses, vegetable and fruit trees. At Gulbarga region, sorghum and pigeon pea crops are cultivated alternatively and endosulfan is largely used in the pest control in pigeon pea. In order to know the residual effect of endosulfan on the growth and biochemical constituents in sorghum, the present work is carried out.

Materials and Methods

Healthy sorghum (*Sorgum bicolor* L. Moench) seeds of variety JP-1-1 were collected from Agriculture Research Station, Gulbarga. The seeds were surface sterilized with 1% mercuric chloride for one minute. After thorough washing with distilled water, hundred seeds each were soaked in different concentrations of endosulfan solution (0.2, 0.4 and 0.6%) for 6 hr. Distilled water was used in place of endosulfan in control. The soaked seeds were incubated in petridishes lined with moist blotter at 28 ± 2°C in dark. The filter papers lined in petridishes were moistened with respective concentration of endosulfan. The data on seed germination was recorded at an interval of 24 hr for 7 days. However, the shoot and root length was measured on 7th day of incubation. The biochemical constituents such as, chlorophylls (Arnon, 1949), soluble proteins (Lowry *et al.,* 1951) and phenol (Malik and Singh, 1980) were estimated. The concentration of free proline was determined in fresh leaf tissue with acid ninhydrin complex in toluene (Bates *et al*., 1973).

Enzyme extract for guaiacol peroxidase (POD) was prepared by grinding 1.0 g leaf material with 8 ml of chilled 0.1 M potassium phosphate buffer (pH 7.0) in a prechilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant thus obtained was used as enzyme source. The enzyme activity was estimated using the method of Sadasivam and Manickam (1996). The 3.0 ml reaction mixture in the cuvette consists of 10 mM potassium phosphate buffer (pH 7.0), 0.05 ml of 20 mM guaiacol solution, 0.1 ml of enzyme extract and 0.03 ml of 12.3 mM hydrogen peroxide. The reaction mixture was mixed well and ongoing reaction was recorded at every 30 sec at 436 nm up to 3 min against the blank without enzyme. The enzyme activity was expressed as changes in absorbance units.

The polyphenol oxidase activity (PPO) was assayed using the method of Thimmaiah (1999). The reaction mixture containing 3.8 ml of potassium phosphate buffer (0.2 M, pH 6.8) and 1.0 ml of 0.05 M catechol was incubated at 30°C and then initiated the reaction by adding 0.2 ml of enzyme extract. The rate of increase in absorbance was recorded at 410 nm wavelemgth at an interval of 30 sec for 5 min. The enzyme activity was expressed in absorbance units.

Superoxide dismutase (SOD) was estimated according to the method of Dhindsa *et al.* (1981). The 3.0 ml reaction mixture contained 13 mM methionine, 25 μ M Nitro blue tetrozolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer (pH 7.8), 50 mM sodium bicarbonate and 0.1 ml enzyme extract. Reaction was started

by adding 2 μM riboflavin and placing the tubes below the15 W fluorescent lamp for 15 min. Reaction was stopped by switching off the light and covering the tubes with black paper. Tubes without enzyme developed maximal colour. A non-irradiated complete reaction mixture served as a blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as that quantity of enzyme, which reduced the absorbance reading to 50% in comparison with the tubes lacking enzyme. The increase in absorbance in the enzyme was taken as 100 and 50% initially, as equivalent to one unit of SOD activity.

Catalase activity was determined according to the method of Sadasivam and Manickam (1996). 3.0 ml reaction mixture was prepared by adding 50 mM potassium phosphate buffer (pH 7.0), 25 ml of enzyme extract and 15 mM hydrogen peroxide and the absorbance was recorded at 240 nm wavelength against a blank without hydrogen peroxide for every 30 sec up to 3 min. One unit of CAT activity was defined as 1 μmol of H_2O_2 consumed per min.

Amylase activity was estimated according to the method of Bernfeld (1995). 0.5 ml of 1% starch was mixed with 0.5 ml of enzyme extract and incubated at room temperature for 5 min. The reaction was stopped by adding 1 ml of DNS (Dinitro salycilic acid) reagent to each tube and incubated in boiling water bath for 10 min. Each reaction mixture was then diluted to 10 ml using distilled water. The amount of maltose liberated was read at 540 nm wavelength against the blank (DNS added before adding the enzyme). The concentration of maltose liberated was calculated using the standard maltose curve. Unit of amylase is expressed as μg of maltose liberated during 5 min of incubation with 1% of starch.

Protease activity was determined using the method given by Takami *et al*. (1990) where, casein was used as substrate. The reaction mixture in a total volume of 1 ml of 1 % casein in phosphate buffer of pH 7.5 and 0.5 ml of enzyme extract was incubated at 30°C for 30 min. After incubation, the reaction was terminated by adding 2 ml of 10% trichloroacetic acid (TCA). After the separation of unreacted casein precipitate by centrifugation, 1 ml of clear supernatant was taken and mixed with 5 ml of 0.4 M Na_2CO_3 and 0.5 ml of Folin-Ciocalteau's phenol reagent. After 30 min, the absorbance was measured at 660 nm wavelength against blank. One unit of protease activity was defined as the amount of enzyme that released one mg of tyrosine per minute under standard assay conditions. Data presented are means ± standard deviation for three replicates. The data means were compared using Duncan's multiple range test (with significance at $p<0.05$).

Results and Discussion

Three concentrations of endosulfan tested were proved to be inhibitory over seed germination and shoot, root length and biomass in sorghum (Figs. 1, 2, 3). Rajannan and Oblisami (1979) suggested that the interactions between various constituents of effluents are responsible for the inhibition of seed germination and seedling growth.

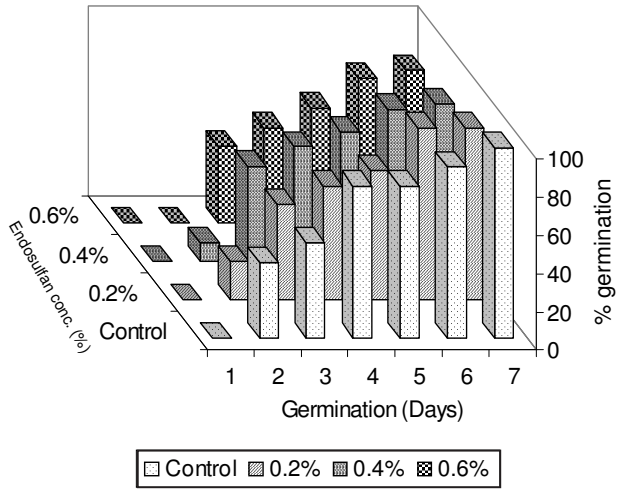

Fig. 1: Effect of three different concentrations of endosulfan on seed germination in sorghum (Data are mean of three replicates)

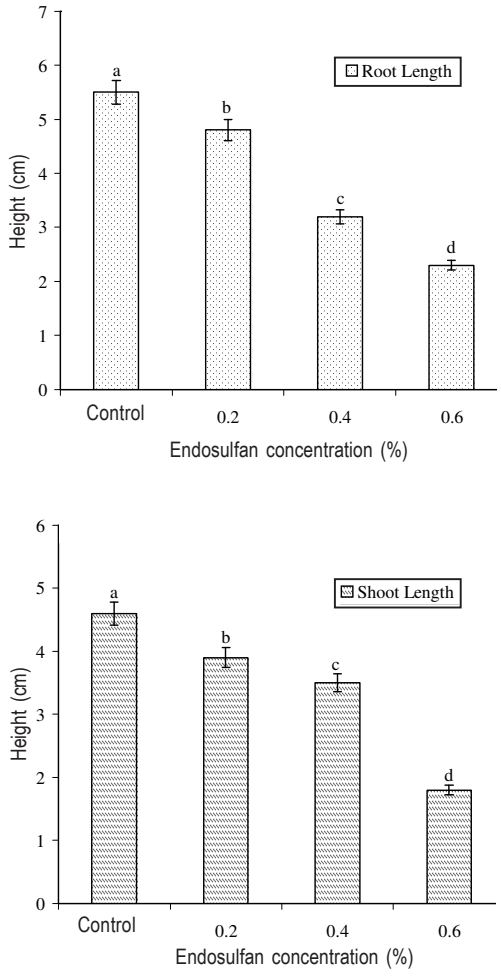

Fig. 2: Effect of three different concentrations of endosulfan on root and shoot length in sorghum. Data are mean ± SD (n=3). Different letters indicate that the mean value is significantly different ($p < 0.05$)

Table - 1: Effect of three different concentrations of endosulfan on the chlorophylls, protein, phenol and proline contents in sorghum seedlings (mg g^{-1}). Values represent the means of three observations ± SD. Different letters indicate that the mean value is significantly different ($p < 0.05$)

Concentration (%)	Chlorophyll-a	Chlorophyll-b	Total chlorophyll	Protein	Phenol	Proline
Control	1.69 ±0.89[a]	1.05 ±0.71[b]	2.75 ±0.54[a]	21.37 ±1.02[a]	1.08 ±1.12[c]	0.18 ±0.35[d]
0.2	1.48 ±0.62[b]	0.97 ±0.39[c]	2.46 ±0.67[b]	20.22 ±1.01[b]	1.11 ±1.09[c]	0.34 ±0.27[c]
0.4	1.07 ±0.75[c]	1.11 ±0.86[a]	2.21 ±0.34[c]	17.31 ±1.12[c]	1.28 ±1.42[b]	0.52 ±0.8[b]
0.6	0.71 ±0.32[d]	1.13 ± 0.58[a]	0.91 ± 0.29[d]	16.0 ± 1.11[d]	1.57 ±1.31[a]	0.98 ±0.07[a]

Table - 2: The effect of three different concentrations of endosulfan on the oxidative enzymes in sorghum seedlings. Values represent the mean of three observations ± SD. Different letters indicate that the mean value is significantly different ($p < 0.05$)

Concentration (%)	Peroxidase (U min^{-1} mg^{-1})	Polyphenol oxidase (U min^{-1} mg^{-1})	Catalase (U min^{-1} mg^{-1})	SOD (U min^{-1} mg^{-1})
Control	18.16 ±0.79[c]	8.23 ±0.09[d]	17.93 ±0.88[a]	7.37 ±0.68[d]
0.2	24.00 ±1.21[a]	11.07 ±0.61[c]	16.39 ±1.28[b]	10.06 ±1.01[c]
0.4	24.25 ±1.05[a]	14.78 ±1.07[b]	14.03 ±1.14[c]	16.36 ±1.41[b]
0.6	23.33 ±0.43[b]	28.00 ± 0.64[a]	10.98 ±0.73[d]	17.51 ±0.94[a]

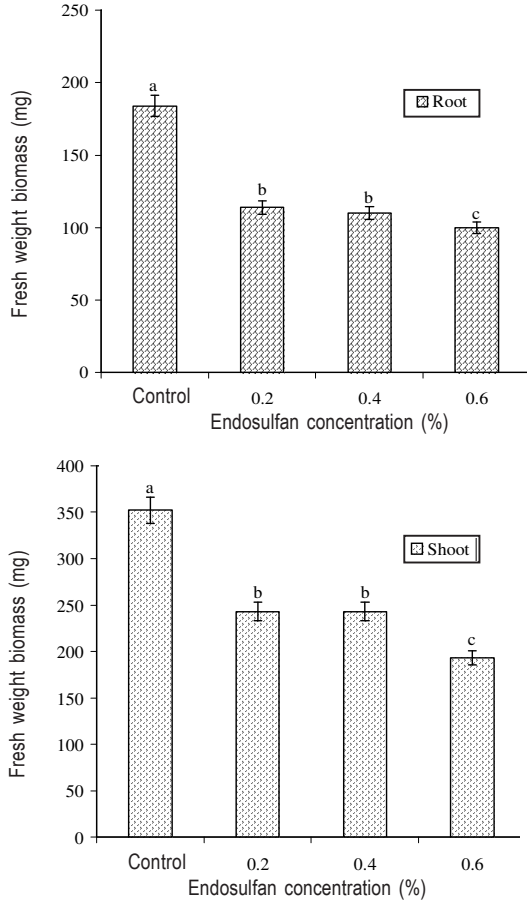

Fig. 3: Effect of three different concentrations of endosulfan on biomass in sorghum. Data are mean ± SD (n=3). Different letters indicate that the mean value is significantly different ($p < 0.05$)

Total chlorophyll and chlorophyll-a content decreased significantly with an increase in endosulfan treatment. However, chlorophyll-b was found increased along with endosulfan concentration (Table 1). This increase in chlorophyll- b may be due to the inter conversion of chlorophyll-a to chlorophyll-b as reported by Ito *et al.* (1996). The loss of chlorophyll content in treatment may be due to the interference in fat metabolism inhibiting root and shoot growth, photosynthesis, nutrient uptake, leaf area, biomass *etc.* (Pandolfini *et al.,* 1992). As the concentration of endosulfan increased, the protein content decreased significantly (Table 1). According to Constantinidou and Kozlowski (1979), the decrease in protein content may be due to the decrease in photosynthesis.

Table - 3: The effect of three different concentrations of endosulfan on the activity of hydrolytic enzymes in sorghum seedlings. Values represent the mean of three observations ± SD. Different letters indicate that the mean value is significantly different ($p < 0.05$)

Concentration (%)	Amylases (U min^{-1} mg^{-1})	Proteases (U min^{-1} mg^{-1})
Control	0.82 ± 0.08[c]	32.60 ±1.93[b]
0.2	2.50 ±0.45[a]	34.10 ±1.73[a]
0.4	1.82 ±0.06[b]	28.80 ±3.41[c]
0.6	0.84 ±0.68[c]	23.90 ±1.49[d]

The increase in phenol content in the seeds treated with endosulfan concentrations (Table 1) is well supported by Agarwal *et al.* (1982). Under stressed conditions increase in the amount of phenols appear to be accompanied by an enhancement in the peroxidase activity, causing the destruction of auxins and thereafter retardation in the growth (Pandolfini *et al.,* 1992).

The proline content also increased in the present studies due to the endosulfan treatment (Table 1). Proline accumulated in plants under various stress conditions. The accumulation of proline in plant due to drought and temperature stress is also well documented by Gzik (1996). Proline acts as a hydrophobic protectant for enzymes and sub-cellular organelles (Lerudulier *et al.,* 1994). This helps the plant to tolerate or adapt to the stress condition. It is evident from these studies that an increase in proline content may serve as a mean of protection of plant tissue against stress.

The activity of catalase was gradually decreased in treated sorghum seedlings as compared to control. However, the activity of

other oxidative enzymes such as, polyphenol oxidase and peroxidase was increased along with the concentration of endosulfan (Table 2). The increase in peroxidase activity may be due to the metabolic response to environmental stress (Fang and Kao, 2000). Lee (2002) also reported that the peroxidase activity increased remarkably with Na_2SO_3 treatments. Since peroxidase activity was very high in treated shoots, accumulated H_2O_2 was utilized for various peroxidative polymerization reactions. The superoxide dismutase is a protective antioxidant enzyme gradually increased in treatment in the present study. It helps in preventing both direct toxicity from superoxide free radicals O_2^- and secondary toxicity from OH and H_2O_2 (Wilde and Yu, 1998).

The hydrolytic enzyme activities vary with endosulfan treatment. Endosulfan treated seedlings showed a sudden increase in amylase activity in lower concentration (0.2%), where as in higher concentrations, the activity was found decreasing. Similarly, protease activity was also enhanced at lower dose (0.2%) and decreased from 0.4% concentrations (Table 3). These results are in favour of the results of Sabale and Misal (2000). In contrast to this, Mathur *et al.* (1983) reported stimulation in amylase activity at increasing concentration of Rogar in *Vigna mungo*. The treatment of pesticides may impose an osmotic stress causing damage to membrane structure. Membrane proteins interact with pesticides, which may affect the production of enzyme proteins (Deshapande and Swami, 1990). Lysosomes are broken down resulting in an increased level of several hydrolytic enzymes under stress condition (Vieira de silva, 1969).

In the present study, seed germination, root, shoot biomass and other biochemical constitutions estimated underwent considerable changes with the increase in endosulfan concentration. It characterized the unfavorable situation served as the biochemical indicator during the stress conditions.

References

Agarwal, M., P.K. Mandi and D.N. Rao: Effect of O_3 and SO_2 pollutants separately and a mixture on chlorophyll and carotenoid pigments of *Oryza sativa* L. *Water Air Soil Pollut*., **18**, 449-454 (1982).

Arnon, D.I.: Copper enzymes in isolated chloroplasts. *Plant Physiol*., **24**, 1-15 (1949).

Bates, S.I., R.P. Waldren and I.D. Teare: Rapid determination of free proline for water stress studies. *Plant Soil*, **39**, 205-208 (1973).

Bernfeld, P.: *In*: Methods in enzymology (*Eds.*: S.P. Colowics and N.O. Kalpan). Vol. I, Academic Press, New York. p.149 (1995).

Bhide, M.: Morphological and histopathological studies on the developing oocytes of *Periplaneta americana* treatment, BHC. *Annl. Zool*., **2**, 63-76 (1986).

Constantinidou, H.A. and T.T. Kozlowski: Effect of sulphur dioxide and ozone on *Ulmus amenicana* seedlings. II. Carbohydrates, proteins and lipids. *Can. J. Bot*., **57**, 176-184 (1979).

Deshapande, A.A. and G.S. Swami: Gibberellin sensitivity in sorghum (*Sorghum bicolor* L. Moench) effect of methyl parathion. *Ind. J. Exp. Biol*., **28**, 83-86 (1990).

Dhindsa, R.S., P. Plumb-Dhindsa and T.A. Thrope: Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot*., **32**, 93-101 (1981).

Fang, W. and C.H. Kao: Enhanced peroxidase activity in the leaves in response to excess iron, copper and zinc. *Plant Sci*., **158**, 71-76 (2000).

Gupta, B.M. and C.P.S. Yadav: Effect of BHC dusts on germination of groundnut and moth bean. *Ind. J. Agric. Sci*., **55**, 754-755 (1986).

Gzik, A.: Accumulation of proline and pattern of alfa amino acids in sugar beet plant in response to osmotic water and salt stress. *Environ. Exp. Bot*., **36**, 29-38 (1996).

Huffman, A.H. and P.W. Jacoby: Effect of herbicides on germination and seedling development of 3 native grasses. *J. Range Manage*., **37**, 40-43 (1984).

Ito, H., T. Ohtsuka and A. Tanaka: Conversion of chlorophyll b to chlorophyll a via 7-hydroxymethyl chlorophyll. *J. Biol. Chem.,* **271**,1475-1479 (1996).

Jaiprakash, H. and H. Singh: Effect of isoproturon on germination and early seedling growth of wild canary grass under varying levels of salinity. *Ind. J. Plant Physiol.,* **27**, 206-208 (1984).

Lee, M.Y.: Effect of Na_2SO_3 on the activities of antioxidant enzymes in geranium seedlings. *Phytochem*., **59**, 493-499. (2002).

Lerudulier, D., A.M. Strom, A.M. Dandekar, L.T. Smith, and R.C. Valentine: Molecular biology of osmoregulation. *Science,* **224**,1064-1068 (1994).

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall: Protein measurement with folin- phenol reagent. *J. Biological Chem.,* **193**, 265-275 (1951).

Malik, C.P. and M.B. Singh: *In*: Plant enzymology and histo-enzymology. Kalyani Publishers, New Delhi. p. 53 (1980).

Mathur, A.M., M.H. Siddiqui, D. Mukherji and S.N. Mathur: Influence of Rogar on seed germination and amylase activity in *Vigna mungo. Proc. Nat. Acad. Sci., India-Sect. B. (Biological Science),* **53**, 47-49 (1983).

Mukhopadhya, R.K. and P.U. Dehadrai: Studies on air breathing catfish *Clarias batrachus* under malathion sublethal exposure. *Ind. J. Exp. Biol.,* **18**, 348-352 (1980).

Pandolfini, T., R. Gabbarielli and C. Comparini: Nickel toxicity and peroxidase activity in seedlings of *Triticum aertivum* L. *Plant Cell Environ.,* **15**, 719-725 (1992).

Rajannan, G. and G. Oblisami: Effect of copper factory effluent on soil and crop plants. *Ind. J. Environ. Hlth.,* **21**, 120-130 (1979).

Sabale, Anjali and B.N. Misal: Effect of endosulfan and methyl parathion on hydrolytic enzymes in germinating seeds of jawar. *J. Environ. Biol*., **21**, 29-35 (2000).

Sadasivam, S. and A. Manickam: Biochemical methods. 2nd Edn., New Age International Publishers. pp. 107-109 (1996).

Sarnaik, S.S., P.P. Kanekar, V.M. Raut, S.P. Taware, K.S. Chavan and B.J. Bhadbhade: Effect of application of different pesticides to soybean on the soil microflora. *J. Environ. Biol.,* **27**, 423-426 (2006).

Singh, M.: Relative toxicity of some insecticides to enzymes alkaline Phosphomonstrase from fat body of desert locust, *Schistocerca gregaries*, Forshal. *Ind. J. Biol.,* **14**, 659-682 (1980).

Singh, M.: Effect of some insecticidal on the alimentary canal of surface grasshopper, *Chrotogonus trachypeterus* Blanchard (Pyrgomorphide: Orthoptera). *Ind. J. Entomol.,* **52**, 89-99 (1990).

Takami, H., T. Akiba and K. Horikoshi: Characterization of an alkaline protease from *Bacillus* sp No. AH-101. *Appl. Microbial Biotechnol.,* **33**, 519-523 (1990).

Thimmaiah, S.K.: Standard methods of biochemical analysis, Kalyani Publishers, New Delhi. pp. 248-249 (1999).

Vieira de Silva J.: *In*: Water and plant life: Problems and modern approaches (*Eds.*: O.L. Lange, L. Kappen and E.D. Schulze). Zpringer Verlag, New York (1969).

Wilde, L.G. and M. Yu: Effect of fluoride on suproxide dismutase (SOD) activity in germinating mung bean seedlings. *Fluoride,* **21**, 81-88 (1998).