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EFFECT OF VARIOUS PLANT PRODUCTS ON PLANT GROWTH & DEFENSIVE ENZYMES PRODUCTION IN CROSS AND RAINFUNDIBULIFORM IS L.CHALLENGEINOCULATED WITH FUSARIUMSOLANI

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Abstract:

The use of plant extracts for disease management is gaining worldwide importance and acceptance. Plant origin "biocides" are non-phytotoxic, relatively safe, systemic and easily biodegradable. The present study was undertaken to investigate the effect of some plant extracts for managing wilt disease of crossandra. Biometric parameters of the crossandra plants, showed significant increase in seed germination (%), shoot and root length when treated with extracts of *Adhatodavasica* followed by *Azadirachtaindica.* Plants infected with *Fusariumsolani* when treated with plant extracts induced metabolic changes in plants including induction of antioxidant defensive enzyme. POX and SOD activities were higher in infected seedling treated with *Adhatoda* plant extracts and CAT activities were higher in infected seedlings treated with neem plant extracts.

Key Words: Plant Extract, Germination of Seeds, Antioxidant Activity, POX, CAT & SOD

Introduction:

Crossandra (*Crossandrainfundibuliformis L.*) is a small, evergreen and perennial shrub, grown in India as a commercially important plant for its attractive purple, yellow and saffron coloured flowers. There are 20 - 25 species but only few like *Crossandrainfundibuliformis, Crossandraundulaefolia, C. mucronata and C. subacaulis*are commercially cultivated (Ramakrishna *et al.,* 2014).

Crossandra crop is attacked by many diseases among which fusarial wilt caused by *Fusariumsolani*is the most difficult pathogen to control, causing destruction of the entire plant.The disease can affect the crop at any stage of growth and the characteristics symptoms are sudden drooping of leaves and petioles and black internal discoloration involving xylem and pith. The control of*F.solani* on *C. infundibuliformis* is difficult to achieve because the long survival of the pathogen in the soil as chlamydospores or as dormant mycelium in infected plant debris (Jegathambigai*et al.,* 2009).Use of plant products (used globally as green pesticides) in plant disease management will provide sustainable solution in agriculture byincreasing crop vigour and by beingecofriendly, easily bio-degradable andcheaper Integrated Disease Management. Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing antimicrobial property (Malkhan Singh *et al*., 2012).With this background,studies were carried out to study the effects of various plant products on the seed Germination, and growth parameters of crossandra plants under pot culture condition and to study the changes in the biochemical activity and anti oxidant defensive enzymes in crossandra plants treated with plant products and challenge inoculated with the pathogen.

Methodology:

Effect of Plant Products on Seed Germination and Growth Parameters of Crossandrapre Inoculated With *Fusariumsolani.*

Pot Culture Experiment:

A pot culture study was conducted with sterile garden land soil in completely randomized block design with ten treatments and three replications. Earthen pots of 30 cm dia. were filled with 5 kg of soil. The inoculum of the pathogen multiplied in sand maize medium was mixed with soil one week prior to sowing @ 50 g kg^{-1} of soil. Twenty crossandra seeds (obtained from CIKS, Sirkazhi) were treated with different plant products separately were sown in each pot. Taking the emergence of radical as criterion for recording the germination, seed germination was recorded on the seventh day.Observations were made shoot length and vigour index using the standard procedures on 15^{th} day from the date of sowing. The germination percentage and vigour index were calculated by using the standard formula (IRST, 1966).

Extraction of Antioxidant Defensive Enzymes:

Plant seedlings were homogenized in 100 mM chilled sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (PVP) (w/v) at 4 C. The extraction ratio was 4 ml. buffer for each one gram of plant material. Homogenate was centrifuged at 15,000g for 15 min. at 4 C. Supernatant was used to measure the activities of enzymes. Lowry et al. (1951).

Assay of Peroxidase Activity:

The activity of POX was assayed by the method of Vidhyasekaran, 1997. The reaction mixture 3 ml. consisted of 0.25% (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6 containing 10 mM hydrogen

peroxide H_2O_2). Volume of 100 ml of the crude enzyme extract was added to initiate the reaction which was measured spectrophotometrically (UV–Visible-160A, Shimadzu). The activity was calculated by measuring the ratio at 470 nm/min. = 0.01, which is defined as 1 unit of activity. The specific activity expressed as (IU mg_1protein).

Assay of Catalase Activity:

The activity of catalase was determined by the method of Constabel*et al.* (1995). Enzyme extract (100 ml) was added to 2.9 ml. of a reaction mixture containing 20 mM H_2O_2 and 50 mM sodium phosphate buffer (pH 7.0). The activity of CAT was measured by monitoring the reduction in the absorbance at 240 nm as a result of H_2O_2 consumption. The amount of consumed H_2O_2 was calculated by using a molar extinction coefficient of 0.04 cm_2 lmol_1. Catalase activity was expressed as units $min.^{-1}$ mg^{-1} protein. One unit of enzyme activity was defined as the decomposition of 1 mol of H_2O_2 per min.

Assay of Superoxide Dismutase:

The activity of SOD was assayed by the method of Beauchamp and Fridovich (1971) by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT). The reactionmixture (3ml) contained 40 mM phosphate buffer (pH 7.8), 13 mM methionine, 75l M NBT, 2l M riboflavin, 0.1 mM EDTA and 10 ml of the crude enzyme extract. The test tubes were shaken and placed 30 cm below light source consisting of 15 W fluorescent lamp. The absorbance was taken at 560 nm. The activity of SOD was expressed at unit mg^{-1} protein one unit of SOD activity is the amount of protein required to inhibit 50% initial reduction of NBT under light. Enzyme activity was expressed as (IU mg^{-1} protein).

Results and Discussion:

Effect of Plant Products on Seed Germination and Growth Parameters of Crossandra Challenge Inoculated with *Fusariumsolani:*

In general, the germination percentage of all the treatments were significantly higher than that of inoculated control. From the data obtained from table 1, the seed germination percentage of *A. vasica*(89.67%) followed by *A. indica and J. Carcus*(86.99 & 82.03% respectively) were found to be superior to the uninoculated control which suggests that they possess plant growth promoting substances. *V. negundo, S. emarginatus* and *A. indica* recorded the least germination percentage.

Plant Extracts	Conc. %	Germination (%)	Shoot Length (cm)	Root Length (cm)	Vigour Index
Acalyphaindica	30	73.90_g	6.60_f	4.10_e	790.7_g
Adhatodavasica	30	89.67_b	10.11_b	5.83_a	1429.3_b
Azadirachtaindica	30	86.99_c	7.99_c	4.70_c	1103.9_c
Sapindusemarginatus	30	74.00_g	6.28_g	4.08_e	766.6_h
Vitexnegundo	30	76.90_f	7.02_e	5.37_b	952.8_f
Jatrophacurcas	30	82.03_d	7.64_d	4.54_d	999.1_e
Carbendazim	0.2	94.53_a	12.46_a	5.78_a	1724.2_a
Inoculated Control	-	35.00_h	3.30_h	2.74_f	211.4_i
Uninoculated control		78.05_e	7.89_c	4.67_c	1005.4_d

Observations on biometric characteristics of *Crossandra*revealed that, seedling height as well as root length was maximum in plants treated with extracts of *Adhatodavasica* (10.11 & 5.83 cm) followed by *Azhadirachtaindica* (7.99 & 4.70 cm) and *Jatrophacurcas* (7.64 & 4.54 cm) respectively. The chemical treatment is significantly superior over that of other treatments as well as both inoculated (3.30 & 2.74 cm) and uninoculated control recording (7.89 & 4.67 cm). Similarly the vigour index was maximum in plants treated with *A. vasica* followed by *A. indica, J. Carcus, vitexnegundo* and *Acalyphaindica* in the decreasing order of merit.

Table 2: Effect of selected plant extracts on the activities of antioxidant defensive enzymes in infected and non infected crossandra seedlings

Antioxidants	Treatments	Before Infection	After 7 days of inoculation		After 14 days of inoculation	
			Non Infected	Infected	Non Infected	Infected
POX activity IU min^{-1} mg^{-1} protein	Control	317.7_e	379.41_e	558.7_e	891.5_e	1140.8_e
	Azhadirachtaindica	548.7_d	607.9_a	1475.8_a	1211.3_a	1453.5_d
	Adhatodavasica	797.5_a	571.9_d	712.2_d	1150.3_d	2265.8_a
	Vitexnegundo	687.6_b	583.8_c	1067.5_c	1179.3_c	1899.7_c
	Jatrophacurcas	621.6_c	591.6_b	1253.8_b	1185.4_b	1935.5_b
CAT activity IU min^{-1}	Control	5.7_d	4.97_e	9.5_e	6.1_e	9.5_e
	Azhadirachtaindica	14.0_a	10.4_d	13.9_d	16.2_a	15.9_a
	Adhatodavasica	10.8_c	13.9_a	19.2_a	11.9_d	10.2_d

mg-1 protein	*Vitexnegundo*	11.9_b	11.6_b	16.2_b	14.5_b	12.8_b
	Jatrophacurcas	10.5_c	10.9_c	15.3_c	13.8_c	12.2_c
SOD activity IU min-1 mg-1 protein	Control	34.2_e	30.97_e	46.0_e	35.7_e	43.28_c
	Azhadirachtaindica	51.7_a	47.19_d	66.39_a	58.1_a	53.5_a
	Adhatodavasica	46.6_d	53.81_a	53.39_d	43.5_d	51.9_a
	Vitexnegundo	49.9_b	50.32_b	60.22_b	52.8_b	51.1_a
	Jatrophacurcas	47.4_c	48.22_c	58.65_c	51.5_c	49.4_b

Effect of Selected Plant Extracts on the Activities of Antioxidant Defensive Enzymes in Infected and Non Infected Crossandra Seedlings:

Crossandra seedlings treated with plant extracts and challenge inoculated with *Fusariumsolani* showed significant increase in the enzyme activities of peroxidase, catalase and superoxide dismutase after 7 days of treatment when compared to control and the results are given in Table 2. *Adhatodavasica* extract treatment caused a higher POX activity (797.5 IU min^{-1} mg^{-1}protein) than *A. indica* treatment (548.7 IU min^{-1} mg^{-1} protein) before the initiation of the infection. Immediately after infection *Azadirachtaindica, Jatrophacurcas* and *Vitexnegundo* showed maximum activity but after 14^{th} day of infection *Adhatodavasica* recorded the maximum POX aactivity.

In contrast CAT activity in *A. vasica* treated crossandra seedlings was lower (10.8 IU min^{-1} mg^{-1}protein) than in crossandra seedlings treated with *V. negundo*(11.9) and *A. indica* (14.0 IU min^{-1} mg^{-1}protein) before infection, Whereas on the 14^{th} day after infection *A. indica*followed by*V. Negundo* and *Jatrophacurcas* recorded maximum CAT activity in the ddecreasing order of merit.

The SOD activity in non infected plants on the 14^{th} days treated with *A. indica*, *V. negundo*, *Jatrophacurcas* and *A. vasica* extracts treatments caused significantly similar elevation (53.5, 52.8, 51.5 & 43.5 IU min^{-1} mg^{-1}protein) respectively, but the infected plant treated with *A. vasica* recorded a sudden increase of SOD activity. The results indicated that infection with *Fusariumsolani* caused a significant increase in the activities of POX, SOD and CAT in crossandra seedlings after 7 and 14 days of infection compared to non infected seedlings in control group.

These data clearly suggested that *Azadirachtaindica*, *Adhatodavasica* and *Vitexnegundo* aqueous extracts increases the resistance level of crossandra plants and prevent *Fusariumoxysporum* disease development through a mechanism involved activation of antioxidant defensive enzymes. Moreover, it could be concluded that the aqueous extracts caused a coordinated activities of H_2O_2 releasing enzymes i.e. SOD and H_2O_2 scavenging enzymes i.e. POX and CAT.

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