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Effect of Bio Agents, Organic Amendments, Silver Nano Particles and Fungicide on Induction of Peroxidase and Poly Phenol Oxidase in Crossandra (*Crossandra infundibuliformis*) Plants Affected by *Fusarium incarnatum* (Desm.) Sacc

***Mallaiah B.*[1*] *and Muthamillan M.*[2]**
[1]*Senior Scientist (Plant Pathology), Maize Research Centre, ARI, PJTSAU-HYDERABAD (Telangana), India.*
[2]*Professor and Head, Department of Plant Pathology, AC & RI, Madurai, TNAU (Telangana), India.*

(Corresponding author: Mallaiah B.)*
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ABSTRACT: Laboratory studies were carried with a objective to study the effect of different bioagents, organic amendments, silver nanoparticles and fungicide in management of crossandra wilt caused by *Fusarium incarnatum* and nematode *Pratylenchus delattrei* and role of enzymes Peroxidase and poly phenol oxidase in offering disease resistance. During the study observations are made on induction of Peroxidase and poly phenol oxidase levels in crossandra plants. All the treatments applied recorded increased levels of PO and PPO in crossandra plants, but among all soil application (SA) of *Trichoderma viride*(T.v) @ 2.5 kg/ha at 20 DAP(days after planting) plus soil drenching (SD) of carbendazim @ 0.1% at 30 DAP plus SA of *T. viride* @ 2.5 kg/ha at 50 DAP plus Foliar application (FA) of *Pseudomonas fluorescens*@ 1.0 kg/ha at 70 DAP plus Foliar application (FA) of *Bacillus subtilis* @ 1.0 kg /ha at 90 DAP was found to be significantly best in induction of PO and PPS in crossandra plants. The same results are also noticed in later stage in reducing disease incidence compare to control and other treatments.

Keywords: Crossandra, *Fusariu* spp, Peroxidase, Poly phenol oxidase.

INRODUCTION

Crossandra mainly grows in southern part of India. Tamil Nadu, Karnataka, Andhra Pradesh and Maharastra are important states growing Crossandra. This crop can be cultivated very easily by small farmers throughout the year.

The flowers are MAJORLY used for hair adornment along with Jasmine flowers. Even though flower are not much fragrant, these flowers are very popular because of their attractive bright colour, light weight and long keeping quality. The flowers are used for making garlands, either alone or in combination with jasmine flowers. Steady market demand as well as guaranteed and regular income have made crossandra a profitable venture for south Indian farmers.

Fungai, bacteria and nematodes are major pathogens that causes diseases in crossandra plants. Among the various fungal diseases wilt disease caused by *Fusarium* spp. is a major problem in Crossandra production and restrict the crop cultivation and is also associated with nematode such as *Pratylenchus delettrei* (Srinivasan and Muthukrishnan 1975). Control of this disease has become very difficult due to its soil borne and complex nature.

Integrated disease management involves use of different individual methods like use of biological agents/chemicals/bio technological/Physical and cultural methods for management of plant diseases. In all the methods of disease control, activation of defense mechanisms in plants are very important for effective disease management. Kamalakannan (2004) reported that soil application of bio control agents such as *Trichoderma* and *P. fluorescens* induced higher amount of Peroxidase, Polyphenol oxidase, Phenylalanine ammonia - lyase and total phenols in coleus plants. Bradley *et al*. (1992) also reported that the increased levels of peroxidase (PO) activity has been correlated with resistance in many plant such as barley, cucurbits, cotton, tobacco and wheat. Usually polyphenol oxidase accumulates upon wounding in plants. Saravanakumar (2002) noticed different isoforms *viz.*, PPO1, PPO2, and PPO3 in PGPR treated plants after inoculation with *Macrophomina phaseolina.* Fuerst *et al.* (2014) reported the role PO and PPO IN biochemical seed defense mechanism. Tyagi *et al.* (2000) clearly explained the role of peroxidase and polyphenol oxidase isozymes in wheat resistance to *Alternaria triticina*

Keeping all in view, pot culture experiments were conducted to observe the changes in induction of defense enzymes in treated crossandra plants with bio agents, organic amendments, silver nano particles and fungicide.

MATERIALS AND METHODS

The local crossandra cultivar used in all pot culture studies. The pathogen (*F. incarnatum*) multiplied on sand maize medium was incorporated in the pots @ 3 per cent (w/w) and nematode inoculated @ 1 nematode per gram of soil. All the treatments like bio agents, nano particles, organic amendments and chemicals were applied as per schedule. The observations on enzyme studies were carried out after last application as mentioned in the treatment schedule. The leaves were collected from the pots at 0, 3, 5, 7 and 9 days after last application in each treatment. The collected leaves were washed several times with sterile distilled water before enzyme extraction. Every treatment was replicated six times with each replication containing three pots with two plants in each pot.

Peroxidase (PO) Assay. One gram of fresh leaf taken from each treatment was ground in one ml of 0.1M phosphate buffer with pH 7.0 in a pre cooled pestle and mortar separately. The homogenate was centrifuged at 15,000 rpm at 4°C for 15 minutes. The supernatant taken from the leaves of each treatment was used as enzyme source. The reaction mixture consists of 1.5 ml of 0.05M pyrogallol, 0.1 ml of enzyme extract and 0.5 ml of one per cent H_2O_2. The changes in absorbance of the reaction mixture was recorded at 420 nm at 30 seconds interval for three minutes at room temperature (28 ± 2°C). The boiled enzyme preparation used as check. The enzyme activity was expressed as change in absorbance of the reaction mixture $min^{-1}g^{-1}$ of leaf (Hammerschmidt *et al.*, 1982).

Polyphenol oxidase (PPO) Assay. One gram of fresh leaves collected from each treatment was ground in one ml of 0.1 M sodium phosphate buffer (pH 6.5) separately. The centrifugation carried at 15,000 rpm for 15 min at 4°C and the supernatant used as the enzyme source. The reaction mixture consisted of 1.5 ml of 0.1M sodium phosphate buffer pH 6.5 and 0.1 ml of the enzyme extract. The reaction initiated by the addition of 0.2 ml of catechol (0.01M). The enzyme activity expressed as change in absorbance at 495 nm at 30 sec interval for three min. The enzyme activity also expressed as change in absorbance per minet per g of leaf (Mayer *et al.*, 1965).

The details of treatment schedule are as follow

T_1	SA of *P. fluorescens* (Pf-18*)* @ 2.5 kg/ha at 20 DAP + Module A
T_2	SA of *T.v* (Tv-9) @ 2.5 kg/ha at 20 DAP + Module A
T_3	SA of *B. subtilis* (Bs-10) @2.5 kg/ha at 20 DAP + Module A
T_4	SA of Neem cake @ 250 kg/ha at 20 DAP + Module A
T_5	SD of carbendazim @ 0.1% at 20 DAP + Module A
T_6	SA of Phorate10G @10 kg/ha at 20DAP + Module A
T_7	FA of nano particles @ 800 ppm at 20 DAP + Module A
T_8	SA of *P.f* (Pf-18*)* @ 2.5 kg/ha at 20 DAP + Module B
T_9	SA @ *T.v* (Tv-9) @ 2.5 kg/ha at 20 DAP + Module B
T_{10}	SA of *B.s* (Bs-10) @ 2.5 kg/ha at 20 DAP + Module B
T_{11}	SA of Neem cake @ 250 kg/ha at 20 DAP + Module B
T_{12}	SD of carbendazim @ 0.1% at 20 DAP + Module B
T_{13}	SA of Phorate10G @10 kg/ha at 20DAP + Module B
T_{14}	FA of nano particles @ 800 ppm at 20 DAP + Module B
T_{15}	SA of *P.f* (Pf-18*)* @ 2.5 kg/ha at 20 DAP + Module C
T_{16}	SA *T.v* (Tv-9) @ 2.5 kg/ha at 20 DAP + Module C
T_{17}	SA of *B.s* (Bs-10) @ 2.5 kg/ha at 20 DAP + Module C
T_{18}	SA of Neem cake @ 250 kg/ha at 20 DAP + Module C
T_{19}	SD of carbendazim @ 0.1% at 20 DAP + Module C
T_{20}	SA of Phorate10G @10 kg/ha at 20DAP + Module C
T_{21}	FA of nanoparticles @ 800 ppm at 20 DAP + Module C
T_{22}	Inoculated control (Pathogens and nematode)

Module A.= Foliar application (FA) of nano particles @ 800ppm at 30 DAP, SA of *T. viride* (Tv-9) @ 2.5 kg/ha at 50 DAP + FA of *P.f* (Pf-18*)* @ 1.0 kg/ha at 70 DAP + FA of of *B.s* (Bs-10) @ 1.0 kg/ha at 90 DAP

Module B = FA of carbendazim @ 0.1% at 30 DAP + SA of *T.v* @ 2.5 kg/ha at 50 DAP and FA of *P.f* (Pf-18*)* @ 1.0 kg/ha at 70 DAP + FA of *B.s* (Bs-10) @ 1.0 kg/ha at 90 DAP

Module C= SD of carbendazim @ 0.1% at 30 DAP + SA of *T.v* @ 2.5 kg/ha at 50 DAP + FA of *P.f* (Pf-18*)* @ 1.0 kg/ha at 70 DAP + FA of *B.s* (Bs-10) @ 1.0 kg/ha at 90 DAP

RESULTS AND DISCUSSION

Induction of Peroxidase (PO) activity: The activity of peroxidase was increased in crossandra plants inoculated with *F. incarnatum* and *P. delattrei* followed by treated with bioagents, organic amendments, nano particles and chemicals in different combinations. The results reveales that the activity of PO was significantly higher in crossandra plants treated with T_{16} (0.513 changes in absorbance /min/g of leaf tissue) at five days after last application, whereas no significant difference was observed in the PO activity in the un inoculated plants throughout the period of study (Table 1).

Table 1: Induction of Peroxidase activity in crossandra plants treated with integration of different combinations of bioagents, organic amendments, nanoparticles and chemical on the management of wilt in pot culture experiment.

Sr. No.	Name of the treatment	*Changes in absorbance /min/g of leaf tissue** Days after last application					%Disease incidence
		0	3	5	7	9	
1.	T_1	0.333	0.431	0.482	0.450	0.431	58.3(49.79)*
2.	T_2	0.354	0.467	0.503	0.483	0.452	50.0(44.98)
3.	T_3	0.330	0.435	0.480	0.446	0.430	61.1(51.41)
4.	T_4	0.327	0.420	0.467	0.440	0.428	63.9(53.06)
5.	T_5	0.350	0.435	0.475	0.472	0.443	52.8(46.58)
6.	T_6	0.345	0.460	0.493	0.470	0.448	63.9(53.06)
7.	T_7	0.347	0.451	0.482	0.463	0.445	55.6(48.18)
8.	T_8	0.336	0.435	0.480	0.448	0.433	36.1(36.92)
9.	T_9	0.340	0.465	0.500	0.481	0.446	30.6((33.54)
10.	T_{10}	0.328	0.430	0.475	0.440	0.432	36.1(36.92)
11.	T_{11}	0.322	0.419	0.465	0.436	0.425	44.4(41.79)
12.	T_{12}	0.342	0.432	0.465	0.462	0.440	33.3(35.24)
13.	T_{13}	0.340	0.455	0.485	0.463	0.447	41.7(40.18)
14.	T_{14}	0.352	0.450	0.478	0.462	0.441	36.1(36.92)
15.	T_{15}	0.330	0.435	0.490	0.455	0.432	27.8(31.79)
16.	T_{16}	0.332	0.469	0.513	0.490	0.460	16.7(24.08)
17.	T_{17}	0.339	0.439	0.483	0.450	0.430	19.4(26.15)
18.	T_{18}	0.340	0.428	0.470	0.449	0.425	22.2(28.11)
19.	T_{19}	0.329	0.445	0.490	0.471	0.440	16.7(24.08)
20.	T_{20}	0.345	0.456	0.494	0.476	0.448	19.4(26.15)
21.	T_{21}	0.333	0.452	0.485	0.465	0.449	22.2(28.11)
22.	T_{22}	0.337	0.345	0.339	0.330	0.329	75.0(60.04)
23.	T_{23}	0.210	0.217	0.215	0.212	0.209	**2.73(0.627)**

* Mean of three replications
DAP – Days after planting

CD (P=0.05)

Treatments	0.03
Days	0.01
Treatments × Days	0.06

Peroxidase is a component of an early response in plants to pathogen attack and plays a important role in the biosynthesis of lignin which ristricts the extent of pathogen spread. The products of this enzyme in presence of hydrogen donor and hydrogen peroxide has antimicrobial and antiviral activity (Van Loon and Callow 1983). Increased levels of peroxidase has been observed in a number of resistant interaction involving plant pathogenic fungi, bacteria and virus (Chen *et al*., 2009; Nandhakumar *et al*., 2001; Kavitha *et al*., 2005).

In the present study, peroxidase activity was two times greater than the un inoculated control. Increased activity of cell wall bound peroxidase has been reported in different plants such as cucumber (Chen *et al*., 2009), rice (Reimers *et al.*, 1992), and tomato (Mohan *et al*., 1993). Increased activities of PO was also observed in *P. chlororaphis* isolate (BCA) and *B. subtilis* isolate (CBE4) treated hot pepper seedlings after challenging inoculation with the pathogen *P. aphanidermatum* (Nakkeeran *et al*., 2006).

The application of endophytic microbes like *B. subtilis* and *P. fluorescens*, alone or in combination in green house and field experiments were found to be effective in managing the chilli *Fusarium* wilt by inducing systemic resistance (ISR) as supported by enhanced activities of PO, PPO, PAL, β-1,3-glucanase, chitinase and phenolics. These are involved in the synthesis of phyto alexins, so that promoting the growth of plants (Sundaramoorthy *et al*., 2012). Furthermore, interactions among the biocontrol agents may also have synergistic effects that could induce ISR and promotes the growth of the plants (Latha *et al*., 2009).

Polyphenol oxidase. The results reveales that Polyphenol oxidase activity also reached the maximum at five days after last application. The induction of PPO was almost double times in treated plants than control. The treatment T_{16} recorded the maximum (0.954 changes in absorbance /min/g of leaf tissue) level of PPO activity at 5 days after last application and it was followed by T_{20} recording of 0.923. The PPO activity was slightly increased in the inoculated control, when compared to untreated control (Table 2).

Table 2: Induction of poly phenol oxidase activity in crossandra plants treated with integration of different combinations of bio agents, organic amendments, nanoparticles and chemicals on the management of wilt in pot culture experiment.

Sr. No.	Name of the treatment	Changes in absorbance /min/g of leaf tissue*					%Disease incidence
		Days after last application					
		0	3	5	7	9	
1.	T_1	0.538	0.792	0.884	0.845	0.636	58.3(49.79)**
2.	T_2	0.540	0.811	0.940	0.863	0.712	50.0(44.98)
3.	T_3	0.545	0.698	0.874	0.780	0.642	61.1(51.41)
4.	T_4	0.539	0.680	0.853	0.769	0.635	63.9(53.06)
5.	T_5	0.542	0.790	0.880	0.835	0.645	52.8(46.58)
6.	T_6	0.541	0.800	0.912	0.855	0.704	63.9(53.06)
7.	T_7	0.545	0.794	0.898	0.812	0.702	55.6(48.18)
8.	T_8	0.539	0.784	0.876	0.840	0.632	36.1(36.92)
9.	T_9	0.544	0.798	0.923	0.852	0.698	30.6((33.54)
10.	T_{10}	0.540	0.692	0.870	0.772	0.638	36.1(36.92)
11.	T_{11}	0.539	0.682	0.840	0.758	0.633	44.4(41.79)
12.	T_{12}	0.540	0.784	0.875	0.831	0.640	33.3(35.24)
13.	T_{13}	0.539	0.812	0.910	0.848	0.698	41.7(40.18)
14.	T_{14}	0.538	0.790	0.884	0.808	0.696	36.1(36.92)
15.	T_{15}	0.540	0.794	0.888	0.858	0.645	27.8(31.79)
16.	T_{16}	0.541	0.828	0.954	0.875	0.720	16.7(24.08)
17.	T_{17}	0.543	0.710	0.892	0.871	0.645	19.4(26.15)
18.	T_{18}	0.544	0.684	0.862	0.775	0.640	22.2(28.11)
19.	T_{19}	0.542	0.798	0.894	0.843	0.652	16.7(24.08)
20.	T_{20}	0.540	0.812	0.923	0.864	0.715	19.4(26.15)
21.	T_{21}	0.539	0.802	0.906	0.825	0.723	22.2(28.11)
22.	T_{22}	0.522	0.542	0.557	0.552	0.535	75.0(60.04)
23.	T_{23}	0.437	0.450	0.440	0.445	0.440	**2.73(0.627)**

* Mean of three replications
DAP – Days after planting
CD (P=0.05)

Treatments	0.03
Days	0.01
Treatments × Days	0.06

Polyphenol oxidase, enzyme contain copper which usually accumulates on wounding in plants. Many reports correlated the induction of PPO activity offering resistance in plants (Velazhahan and Vidhyasekaran 1994). The enzymes PO and PPO plays a vital role in catalyzing and the oxidation of phenolic compounds through a PPO-PO-H_2O_2 path way (Srivastava, 1987).

The present study cofirms that the integrated module T_{16} significantly increases the activity of PPO in crossandra leaves. The similar results reported by Kavitha (2004); Kamalakannan (2004) that the applications of *P. fluorescens* isolate and *B. subtilis* isolate B 49 combination significantly increased PPO activity against the soil borne pathogens *Pythim aphanidermatum* and *Macrophomina phaseolina*.

The increase in PPO activity may be due to activation of latent host enzyme, solubilization of host PPO, are due to *de novo* synthesis (Manibhushan Rao *et al.*, 1988). The induced PPO might have involved in offering resistance in crossandra against wilt disease. Ramamoorthy and Samiyappan (2001) reported that treatment of chilli plants with *P. fluorescens* challenge inoculated with *C. capsici* increased PPO activity. Our results are similar with earlier workers that, the strains of *B. subtilis* and *P. fluorescens* were able to induce increased activities of PPO on challenge inoculation with *A. alternata* in watermelon (Uma Maheswari, 2009). Increased PO and PPO activity has been shown in a number of incompatible disease interactions involving plant pathogenic fungi, bacteria and viruses (Chen *et al.*, 2009; Kandan *et al.*, 2002; Saravanakumar *et al.*, 2007). The application of *B. subtilis* and *P. fluorescens,* singly or in combination at green house and field conditions recorded effective in control of chilli *Fusarium* wilt as evidenced by enhanced activities of PO, PPO, PAL, β-1,3-glucanase, chitinase and phenolic involved in the synthesis of phytolaexins (Sundaramoorthy *et al*., 2012). The levels of PO and PPO in treated plants are associated in importing resistance in plants. Among the twenty two treatments with different combinations tested, the treatment of T_{16} recorded least per cent disease incidence (2.8%) with 96.5 per cent disease reduction over control indicating the role of PO and PPO in disease management. Saberi *et al.*, (2021) reported the activity of polyphenol oxidase, and

peroxidase in some wheat genotypes against take-all disease. Naz *et al.* (2021) also reported the induction of defense-related enzymes (PO & PPO) and enhanced disease resistance in maize against *Fusarium verticillioides* by seed treatment with *Jacaranda mimosifolia*. Taha, *et al.* (2021) also noticed the increased levels of peroxidase and polyphenol oxidase in tomato plants treated with soil Streptomyces isolates and induction of plant resistance against tomato mosaic virus. Liang *et al.* (2017). Observed increased activities of peroxidase and polyphenol oxidase enhance cassava resistance to mite *Tetranychus urticae*. Gopalakrishnan *et al.* (2021). Also got similar results with *Streptomyces* spp. and host-plant resistance induction against charcoal rot of sorghum.

CONCLUSION

It was found that SA of *T. viride* @ 2.5 kg/ha at 20 DAP plus SA of carbendazim @ 0.1% at 30 DAP plus SA of *T. viride* @ 2.5 kg/ha at 50 DAP plus FA of *P. fluorescens@* 1.0 kg/ha at 70 DAP plus FA of *B. subtilis* @ 1.0 kg /ha at 90 DAP was found to be significantly superior in increase of defense enzymes like PO and PPOs. These enzymes also offering resistance in later stage by preventing disease incidence in plants and signifies their role as important component of disease resistance in plants against pathogens including insects.

Conflict of Interest. None.

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