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INDUCTION OF DEFENSE-RELATED ENZYMES IN ONION BY USING COMBINED APPLICATION OF FUNGAL AND BACTERIAL BIOCONTROL AGENTS WITH AM FUNGI AGAINST *FUSARIUM OXYSPORUM* F. SP. *CEPAE*

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Abstract

Basal rot of onion is one of the most destructive disease, in different onion growing regions of Tamil Nadu, India which caused by *Fusarium oxysporum* f.sp. *cepae*. Efficacy of various biocontrol agents and AM fungi was evaluated for the potential to manage the basal rot of onion *in vitro* pot culture experiment. The experiment clearly indicate that the plant treated with consortial formulation of TV5+PF2+*G. mosseae* showed higher induction of defense enzymes *viz*., peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase and phenol.

Key words: onion; basal rot; *F. o.* f.sp. *cepae*; defense enzymes

Introduction

Onion (*Allium cepa* var. *aggregatum* G. Don) is a major vegetable crop grown in India which is known as (''Queen of the kitchen''). It belongs to the family *Alliaceae*. The crop is originated in Central Asia (Baloch, 1994). In world, India ranks first in area and second in onion production (Kumar *et al.*, 2015). India has about 1.2 million hectare area under onion cultivation constituting about 10% of total acreage under vegetable with an annual production of 19.40 MT in 2015 (Singh *et al*., 2017). The total area under production of onion in India during 2016-2017 was 1306 thousand ha with 22427 thousand MT production. However, in Tamil Nadu the total area was 239.286 thousand ha with total production 6559.8 thousand MT/ha (NHB 2016). The basal rot disease of onion caused by *Fusarium oxysporum* f.sp. *cepae* (Hans) is the most destructive disease and causes yield losses in all growing areas of the world (Coskuntuna and Ozer, 2008). Onion basal rot was first observed in Ohio, USA (Clinton, 1915). In India, the occurrence of this disease was first reported from Rajasthan (Ilhe *et al*., 2013). In Tamil Nadu, this disease was first observed by Ramakrishnan and Eswaramoorthy (1982) from Coimbatore district. Onion basal rot considered as one of the most important soil-borne diseases of onion, causes severe losses in productivity both in the field and in storage condition (Coskuntuna and Ozer, 2008). Yield loss up to 50 per cent has been recorded in susceptible cultivars (Everts *et al*., 1985) with 90 per cent losses during the seedling stage. Biological control using fungal and bacterial antagonists has been suggested as a possible control of *Fusarium* basal rot disease of onion (Coskuntuna and Ozer, 2008). Several researchers have observed improved disease control using various biocontrol organisms such as Trichoderma sp. (Adekunle *et al*., 2001) and *Pseudomonas* sp. (Rashmi Srivasta *et al*., 2010) as they have antifungal, plant growth promoting and plant defense inducing activities (Zaidi *et al*., 2004). Besides these organisms, the Arbuscular mycorrhizal fungi (AMF) have also been reported in combating the soil-borne diseases by inducing plant defense proteins like PR proteins (Van Loon *et al*., 2006).

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Materials and methods

Induction of defense enzymes and phenol in onion plant treated with antagonist and *G. mosseae*

challenge inoculated with F. oxysporum f.sp. cepae (pot culture)

Sterilized soil was mixed with the pathogen inoculum @5 percent (W/W) level and filled in 30cm earthen pots. The most effective soil application dosages identified in earlier experiment alone were used for testing the efficacy of soil application of the antagonists (Tv_3, Pf_2 and *G. mosseae*). The antagonists meant for soil application were applied to the pots and incorporated well. The onion bulbs were planted in pot soil mixed with the inoculum of test pathogen alone served as a control. Carbendazim @0.1% was used for comparison. The experiment was conducted with three replications in a randomized block design with five bulbs per pot. All the observations *viz.*, defense enzymes and phenolic content of onion were recorded.

Enzyme extraction

One g of fresh leaf tissue was homogenized with 2 ml of 0.1M sodium citrate buffer (pH 5.0) at 4°C. The homogenate was centrifuged for 20 min. at 10,000 rpm. Enzyme extracted in 0.1 M sodium phosphate buffer (pH 7.0) was used for the estimation of Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase (PAL). Enzyme extract was stored in deep freezer (-70°C) until used for biochemical analysis.

Assay of Peroxidase (PO)

Peroxidase activity was assayed as per the procedure described by Hammerschmidt *et al*., (1982). The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01 M sodium phosphate buffer, pH 6.0 and 0.1 M hydrogen peroxide. Enzyme extract (0.1ml) was added to initiate the reaction, which was followed colorimetrically at 470 nm. Crude enzyme preparations were diluted to give changes in absorbance at 470 nm of 0.1 to 0.2 absorbance units/min. The boiled enzyme preparation served as blank. Activity was expressed as changes in absorbance at 470 nm min^{-1} g^{-1} of fresh tissue.

Assay of Polyphenol Oxidase (PPO)

Polyphenol oxidase activity was determined as per the proce-dure given by Mayer *et al.*, (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 ml of the enzyme extract. To start the reaction, 0.01 M catechol was added and the activity was expressed as changes in absorbance at 470 nm min^{-1} g^{-1} of fresh tissue.

Assay of Phenylalanine ammonia lyase (PAL)

The PAL activity was assayed as per the method described by Ross and Sederoff (1992). The assay mixture containing 100 µl of enzyme, 500 µl of 50 mMTrisHCl (pH 8.8) and 600 µl of 1 mM L-phenylalanine was incubated for 60 min. and the reaction was arrested by adding 2 N HCl. Later 1.5 ml of toluene was added, vortexed for 30 sec., centrifuged (1000 rpm, 5 min.) and toluene fraction containing transcinnamic acid was separated. The toluene phase was measured at 290 nm against the blank of toluene. Standard curve was drawn with graded amounts of cinnamic acid in toluene as described earlier. The enzyme activity was expressed as nmoles of cinnamic acid min^{-1} g of fresh tissue.

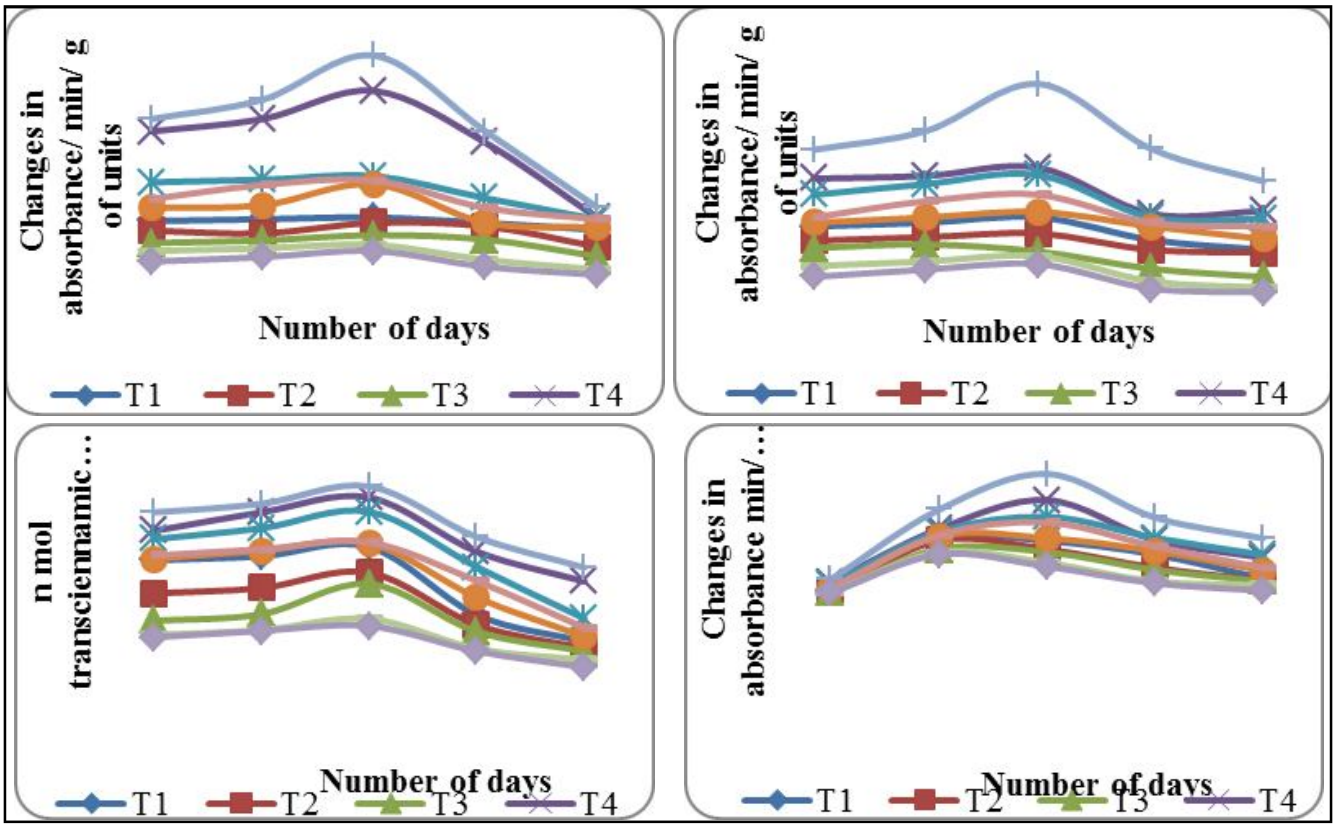
Assay of Phenol

Phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993). One gram of fresh tissue was homogenized in 10 ml of 80 per cent methanol and agitated for 15 min. at 70°C. One ml of the methanol extract was added to 5 ml of dist. water and 250 µl of Folin Ciocalteau reagent (1N) and the solution was kept at 25°C. After three min., one ml of saturated solution of Na_2CO_3 and one ml of dist. water was added and the reaction mixture was incubated for 1 h. at 25°C. The absorption of the developed blue colour was measured using a spectrophotometer at 725 nm. The content of the total soluble phenols was calculated according to a standard curve obtained from a Folin-Ciocalteau reagent with a phenol solution (C_6H_5OH) and expressed as catechol equivalents g^{-1} of fresh tissue.

Results

Among the various treatments, the combination treatment (T_7) involving soil application of *T. viride* (2.5kg/ha), *P. fluorescens* (2.5kg/ha) and *G. mosseae* (12.5kg/ha) recorded higher peroxidase (2.82), polyphenol oxides (2.46) and phenylalanine ammonia (100.42) activities on 5th day when compared with other treatments. This was followed by T_4 (2.42, 1.53 and 95.63), T_5 (1.45, 1.23 and 89.42) and T_8 (1.40, 1.23 and 76.20) treatments in the decreasing order of merit respectively. The maximum PO, PPO and PAL activity was observed on the 5th day in all the treatments and thereafter a gradual decrease was observed. The healthy control plants also showed increase in enzyme activity upto five days and thereafter showed decline (Fig. 1, 2 and 3).

With regard to phenol, the results revealed increased activity of phenolics due to treatment with Tv_3, Pf_2 and *G. mosseae* and challenge inoculation with the pathogen. Among the treatments, soil application of *T. viride* (2.5kg/ha), *P. fluorescens* (2.5kg/ha) and *G. mosseae* (12.5kg/ha) (T_7) recorded higher phenolics activity when compared to other treatments. This was followed by T_4 (155.07), T_5 (143.22) and T_8 (139.17) treatments in the decreasing order of merit. The maximum phenolics activity



T_1- SA of *T. viride* @ 2.5kg/ha; T_2- SA of *P. fluorescens* @ 2.5 kg/ha; T_3- SA of *G. mosseae* @ 12.5kg/ha; T_4- SA of *T. viride* @2.5kg/ha + SA of*P.fluorescens* @ 2.5kg/ha; T_5- SA of *T. viride* @ 2.5kg/ha + SA of *G. mosseae* @ 12.5kg/ha; T_6 - SA of*P.fluorescens* @ 2.5kg/ha + SA of *G. mosseae* @ 12.5kg/ha; T_7- SA of *T. viride* @ 2.5kg/ha + SA of *P. fluorescens* @2.5kg/ha + SA of *G. mosseae* @ 12.5kg/ha; T_8- Carbendazim 50% WP@0.1%; T_9- Inoculated control ; T_{10}- Un inoculated control.

(173.54) was observed on the 5th day and thereafter a gradual decrease was observed. The healthy control plants also showed slight increase in phenolics activity up to three days and thereafter showed decline (Fig. 4).

Discussion

Similar findings were resulted in green gram plants treated with a talc based formulation containing Pf1+Tv1 (Thilagavathi *et al.*, 2007). KhiroodDoley *et al*., (2014) reported that the highest peroxidase activity was observed in combined application of AM fungi and *Trichoderma* in presence of *S. rolfsii*in groundnut followed by single application of AM fungi or *Trichoderma*. Application of *P. fluorescens* along with azoxystrobin showed 'twofold' increase in activities of PPO (Anand *et al*., 2010). Chitin supplemented *Trichoderma* treated plants challenged with *R. solani* showed increased accumulation of polyphenoloxidase (Solanki *et al.*, 2011). Application of *T. harzianum* enhanced induction of defense related enzyme PPO which may be the effective defense mechanism shown by the plants against *Alternaria* leaf spot of sesame (Lubaina and Murugan, 2015).

A higher activity of PAL was observed in *P. chlororaphis* pretreated chilli seeds challenge inoculated with *P. aphanidermatum* (Kavitha *et al.*, 2005). Similarly, cotton seeds treated with endophytic *Bacillus* species challenged with *R. solani* resulted in increased activity of PAL (Rajendran and Samiyappan, 2008) and the banana fruits treated with antagonist mixture recorded maximum induction of phenylalanine ammonia lyase (Sangeetha *et al.*, 2010). PAL is a key enzyme in the first stage of phenyl propanoid metabolism leading to the synthesis of lignin, phenols, phytoalexins, and other compounds involved in a localized plant resistance process (El-Beltagi *et al*., 2012). Banana fruits treated with a mixture of biocontrol agents and challenge inoculated with *L. theobromae* and *C. musae*, recorded three fold increased in phenolic content (Sangeetha *et al.*, 2010). Treatment with *T. harzianum* and challenge inoculation of *M. phaseolina* to groundnut plants enhanced the induction total phenol and ortho-dihydric phenol (Sreedevi *et al.*, 2011). These earlier findings were supported for present study.

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