In Vitro Evaluation of Phenolic Compounds against Fusarium Causing wilt Disease of Guava (Psidiumguajava)

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Abstract—*Two phenolics compounds i.e. Phenol and Salicylic acid were tested by poisoned food technique to find out their efficacy against the test pathogens i.e. Fusarium solani and Fusarium oxysporum at 100, 200, 400 ppm. In case of both the pathogens phenol gave most promising results and reduced the mean radial growth by 66.46%, 69.44%and 70.62% and 64.03%, 59.27% and 68.38% at 400 ppm concentration on 3rd, 5th and 7th day after inoculation over control respectively. Therefore salicylic acid was found less effective. Data presented in tables revealed that the value of T_1, T_2 and T_3 were found significantly different from T_0 (control) at level $p<0.05$.*

1. INTRODUCTION

The main toxins produced by *Fusarium* species i.e. fumonisins and trichothecenes. *Fusarium* spp. are predominantly common soil fungi, found in almost all parts of the world as a non-detrimental colonizer of root surface or a weak invader of the root cortex of many plants. There are over 80 known strains only of *F. oxysporum* which show specific pathogenicity to particular crops causing the vascular wilt diseases. In case of guava, wiltingis known as 'Syndrome disease' because of various numbers of symptoms; and pathogens associated with it, which causes deterioration of health of the plant leading to the drying appearance on plant parts and finally death of the plant (Dwivedi and Dwivedi, 1999). Amongst them two species of *Fusarium* (*Fusarium solani* and *Fusarium oxysporum*)are widely reported as most destructive disease of guava (Edward, 1960).This disease is soil-borne and attention is needed to control it. Many scientists have worked towards this step to suggest lots of recommendations from time to time to control the guava wilt. In this context, phenolic compounds have been found one of the most effective chemical means of management to control fusarium population *in vitro*.

Phenolic compounds have one or more aromatic rings contain different hydroxyl groups. The antifungal activity of phenolic compounds both *in vitro* and *in vivo* conditions have been reported by several workers (Dwivedi, 1990; Cherif et al., 2007). Dwivedi (1990) studied the effect of phenolic compounds against *Fusarium* f. sp. *psidii*causing wilt disease in guava. He reported that amongst the four phenolic compounds, salcylic acid and phenols effectively checked the growth of test pathogens, while picric acid and benzoic acid were not so effective. He and Wolyn (2005) reported the induction of salicylic acid and lignification in *Asparagus* following inoculation with both pathogenic and non-pathogenic *F. oxysporum*. Shukla and Dwivedi (2013) tested the antifungal effect of phenolic compounds viz.,salicylic acid, phenol and benzoic acid *in vitro* against *Fusarium udum andF.oxysporum f. sp.ciceri at*0.1% and 0.15% concentration. They found that salicylic acid and phenol were effective at higher concentration only, while benzoic acid was effective at all respective concentrations.

2. MATERIALS AND METHODS

Two phenolics compounds i.e. Phenol and Salicylic acid were tested by poisoned food technique (Grover and Moore 1962) to find out their efficacy against the test pathogens at 100, 200, 400 ppm. One hundred ml Czapex-dox agar medium was autoclaved in 250 ml Erlenmeyer flask at 15 Ib/in^2 pressure for 1 h. Accurate amount of both the chemicals were amended with the cooled down medium separately so as give the final concentration. Agar medium was poured in sterilized Petri dishes and allowed to solidify. After solidification the plates were inoculated with 2 mm disc of fungal pathogen cut from actively growing colony of pure culture plate and incubated at 28 ± 2^0C. Observation on colony diameter was recorded on 3rd, 5th and 6th day after inoculation.

3. RESULTS

Amongst phenolic compounds tested, phenol was found quite effective than salicylic acid against both the pathogens. Data presented in tables revealed that the value of T_1, T_2 and T_3 were found significantly different from T_0 (control) at level $p<0.05$. In case of *Fusarium solani*,phenol effectively reduced the mean radial growth by 66.46%, 69.44%and 70.62% at 400 ppm concentration on 3rd, 5th and 7th day after inoculation over

control followed by salicylic acid and inhibited the growth by 54.49%, 52.68% and 55.43% at same concentration (Table1 Fig1A Plate 1A). In case of *Fusarium oxysporum* phenol significantly reduced the mean radial growth by 64.03%, 59.27% and 68.38% at 400 ppm concentration followed by salicylic acid by 29.37%, 43.54% and 57.91% at same concentration on 3rd, 5th and 7th day after inoculation over controlrespectively (Table 2 Fig1B Plate 1B).

Table 1: In vitro effect of phenolic on mean radial growth (mm) of Fusarium solani at different concentrationon 3rd, 5th and 7th DAI

Treatment	Phenol (C6H5OH)			Salicylic acid (C6H4(OH)COOH)		
DAI	3rd D	5thD	7thD	3rd D	5thD	7thD
T0(control)	21.7±0.38a (00.00)	39.9±0.60a (00.00)	56.1±0.27a (00.00)	21.7±0.38a (00.00)	39.9±0.60a (00.00)	56.1±0.27a (00.00)
T1(100 ppm)	14.9±0.86b (31.18)	26.6±0.08b (33.33)	34.0±0.05b (39.46)	14.2±0.11b (34.70)	28.5±0.32b (28.68)	34.6±0.18b (38.33)
T2(200 ppm)	14.4±0.14c (33.62)	18.5±0.08c (53.52)	24.1±0.18c (56.97)	13.5±0.24c (37.60)	26.0±0.51c (34.83)	33.8±0.08c (39.69)
T3(400 ppm)	7.30±0.20d (66.46)	12.2±0.49d (69.44)	16.5±0.46d (70.62)	9.90±0.05d (54.49)	18.9±0.05d (52.68)	25.0±0.26d (55.43)
CD at p<0.05	2.36	1.31	0.94	0.78	1.427	0.71
SEM	0.71	0.39	0.28	0.23	1.427	0.21

Table 2: In vitro effect of phenolic on mean radial growth (mm) of Fusarium oxysporumat different concentration on 3rd, 5th and 7th DAI

Treatment	Phenol (C6H5OH)			Salicylic acid (C6H4(OH)COOH)		
DAI	3rd D	5thD	7thD	3rd D	5thD	7thD
T0 (control)	22.8±0.11a (00.00)	44.9±0.06a (00.00)	68.4±0.51a (00.00)	22.8±0.11a (00.00)	44.9±0.06a (00.00)	68.4±0.51a (00.00)
T1 (100 ppm)	16.3±0.8b (28.39)	30.6±0.88b (31.74)	44.3±0.55b (35.27)	20.7±0.65b (9.23)	33.4±0.37b (25.51)	47.6±0.68b (30.39)
T2 (200 ppm)	11.9±24.5c (47.65)	24.5±0.38c (45.32)	31.3±0.53c (54.15)	19.6±0.70c (14.05)	31.4±0.23c (30.04)	43.2±0.17c (36.76)
T3 (400 ppm)	8.20±18.3d (64.03)	18.3±0.30d (59.27)	21.6±0.63d (68.38)	16.1±0.26d (29.37)	25.3±0.36d (43.54)	28.8±0.66d (57.91)
CD at p<0.05	1.45	1.67	1.86	1.65	0.95	1.82
SEM	0.44	0.50	0.56	0.50	0.28	0.55

SEM: Standard error mean, Mean of radial growth in (mm). Data are expressed as mean±SE (n=3). Means within the same column and followed by the different letter are significantly different from each other according to Duncan's Multiple Range Test at p<0.05 level of significance. Values in parentheses correspond to % inhibition in the growth of *Fusariumsolani***(Table 1)** and *Fusarium oxysporum***(Table 2)**.

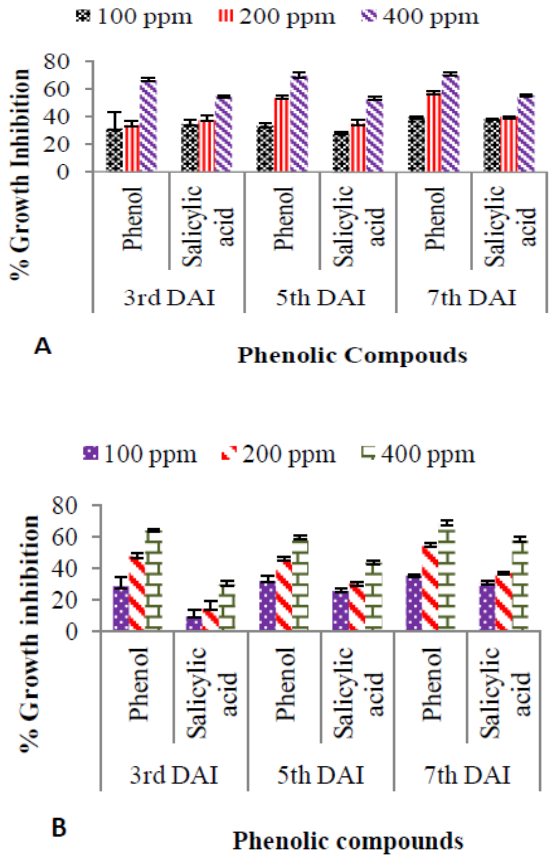

Fig. 1: In vitro effect of phenolic compounds against Fusarium solani(A) and Fusarium oxysporum(B)at different concentration on 3rd, 5th and 7th day after inoculation

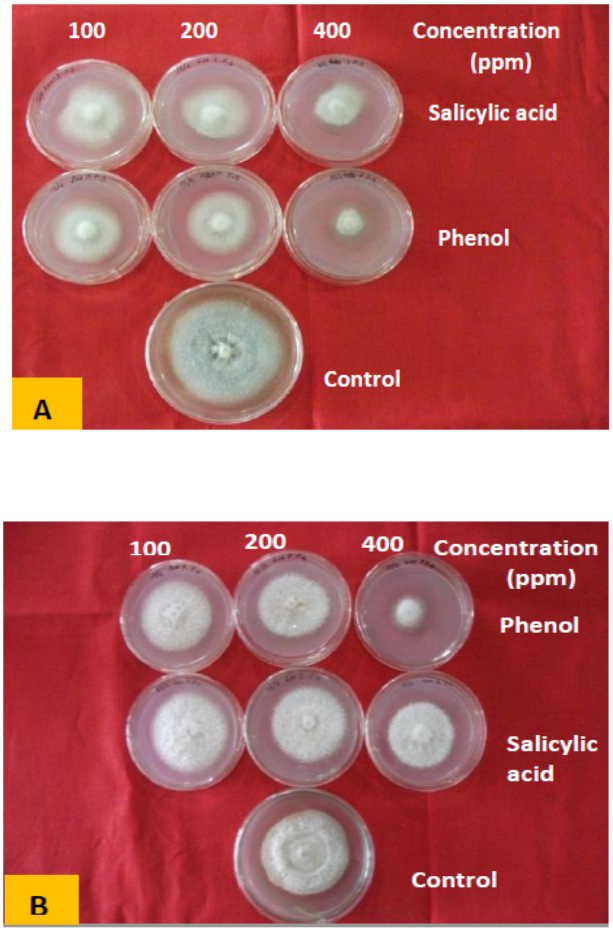

PLATE 1: In vitro effect of Phenolic compoundsagainst Fusarium solani(A) and F. oxysporum(B).

4. DISCUSSION

Phenolic compounds are plants secondary metabolites that constitute one of the most common and widespread groups of substances in plants. Dwivedi (1990) reported that some phenolics i.e. phenol and salicylic acid exerted complete inhibition of *Fusarium* f. sp. *psidii*causing wilt disease in guava at 0.1% and 0.15% concentration, while picric acid and benzoic acid were least effective.

Weidenborner et al. (1990) reported that many flavones and flavanones have been shown to be active against fungal pathogens commonly found during the storage of fruits and vegetables, i.e. *Aspergillus*sp., *Botrytis cinerea*and *F. oxysporum*. The fungicidal activities of a number of stilbenes and related compounds have been tested against several fungiincluding some pathogens which infect grapes during storage.Reddy et al. (1999) reported protection of wheat from *F. graminearum* and synthesis of phenolics, especially ferulic acid, in primary leaves following the treatment of wheat seed with chitosan. Similarly, *Pythiumoligandrum* induces phenolic compounds, particularly ferulic acid, protecting wheat from *F. graminearum* (Takenaka et al., 2003). *In vitro* studies revealed that phenolic compounds extracted from olive plants (*Oleaeuropaea*L.), tyrosol, catechin, and oleuropein showed antifungal activity, thus affecting plant resistance against *Phytophtora*sp. (Del Rio et al., 2003).

Phenolic compounds are well known for their role in plant defense responses, both as preformed inhibitors and those formed in response to attack by pathogens (Nicholson and Hammerschmidt, 1992; Steinkellner et al., 2005). Phenolic compounds causes the vascular browning symptom characteristically associated with *Fusarium* wilt (Mace et al., 1972) as well as many other host–pathogen interactions (Hammerschmidt, 2005). Phenolic compounds are building blocks for many secondary metabolites including those involved in host defense responses. He and Wolyn (2005) reported the induction of salicylic acid and lignification in *Asparagus* following inoculation with both pathogenic and non-pathogenic *F. oxysporum.*

Wu et al. (2009) reported that the allelopathic potential of an artificially applied allelochemical, i.e. benzoic acid against *Fusarium oxysporum*f.sp. *niveum*causing watermelon wilt. They found that benzoicacid strongly inhibited its growth, sporulation and conidial germination whereas it stimulated virulence factorsof this pathogen. The biomass was reduced by 83–96% and the conidia germinating rate and conidiaproduction rate were decreased by 100% at a concentration of >200 mg L^{-1}.

Shukla and dwivedi (2013) tested the efficacy of phenolic compounds and reported that salicylic acid at 0.1% and phenol at 0.15% concentration completely (100%) checks the growth of *Fusarium udum*while benzoic acid at 0.15% concentration reduced the growth by 98.52%. In case of *F. oxysporum f. sp.ciceri*the reduction in growth has been found to be 98.70%, 98.52% and 95% at 0.15% concentration of salicylic acid, phenol and benzoic acid, respectively.

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