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In vitro evaluation of toxic metabolites produced by some foliar pathogens of guava (*Psidium guajava* L.)

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The relative toxin(s) production by the some foliar pathogens, like *Pestalotia psidii* Pat. *Colletotrichum gloeosporioides* Penz, and *Botryodiplodia theobromae* Pat., of guava was studied *in vitro* in Czapek's dox (CDB). modified Fries 3 (MFB) and Richard's synthetic broth (RSB) media. The effect of toxin(s) produced by respective fungal pathogen and their crude toxin potency was evaluated against germinated rice seed (variety IR-36) and on excised guava twigs (variety L-49) at five levels of toxin dilutions at N, N/1, N/2, N/3 and N/4 after 96 and 15 hours respectively. In germinated rice seed strong inhibition in radicle and plumule length was recorded in toxin secreted by *C. gloeosporioides, B. theobromae* and least in *P. psidii* in RSB media. An intense wilting in excised guava twigs was also observed in toxin produced by *C. gloeosporioides, B. theobromae* and least in *P. psidii* in both RSB and MFB media. Almost no wilting was recorded by all pathogens in CDB media in supporting production of toxins in culture. *C. gloeosporioides* had higher ability for toxin production compared to *B. theobromae* and *P. psidii.*

Key words: Toxic metabolites, foliar pathogens, *P. psidii, C. gloeosporioides, B. theobromae,* vigour index

INTRODUCTION

Guava is an important fruit crop grown in tropical and subtropical climate of India. Guava (*Psidium guajava* L.) is susceptible to a number of pathogens among which *P. psidii, C. gloeosporioides* and *B. theobromae* are important. These pathogens produce leaf blight, leaf spot, die back, soft and dry rot symptoms resulting in huge losses at all stages of plant growth and fruit yield (Mishra and Prakash, 1994). It has been established that the host-pathogen interactions are associated with synthesis of host invading substances, amongst which toxin is one of them. Toxin(s) play an important role in pathogenesis, may be produced either by the pathogen or by the host or upon interaction between these two (Sadasivan, 1969). It has been suggested that pathogens of similar nature could secrete toxin under *in vitro* condition (Wang and Li 2002 and Saikia *et al.,* 2004). The present investigation was undertaken to study the ability to produce toxin *in vitro* and their roles on symptoms development in excised guava twigs by some of the foliar pathogens of Guava.

MATERIALS AND METHODS

To study the ability of toxin production by *Pestalotia psidii, Colletotrichum gloeosporioides* and *Botroyodiplodia theobromae,* respective pathogen was grown in 150 ml Borosil conical flask containing 50 ml each of different synthetic medium viz., RSB, MFB and CDB media at 28±1°C. The culture filtrate was extracted twenty days after incubation (Niranjan and Shekhar Shetty, 1998). One hundred healthy rice seed (variety IR-36) were thoroughly washed with 0.1% teepol water followed by repeated washing in running tap water and finally 2-3 rinses in distilled water. The cleaned seeds were subjected to germination test (Narain and Das, 1970) and per

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cent germination of seed was recorded at 96 hrs after incubation. The seeds were germinated in large Petridish over double layer moist filter paper at room temperature. Various dilutions of cell free culture filtrate were prepared as N, N/1, N/2, N/3 and N/4 (N=normal) using sterilized distilled water. Ten ml of each dilution was pipetted in each Petriplate (90 mm) using a clean and sterilized pipette for each dilution separately. Freshly germinated rich seeds (twenty seeds) were placed in to a folded filter paper in each plate. The data on radicle and plumule length of germinated rice seeds were measured ninety-six hrs after incubation (Luke and Wheeler, 1955) and the Vigour index calculated as per formula used by Abdul Baki and Anderson (1973). The per cent inhibition in seed germination, radicle and plumule length was calculated as per methods followed by Vidyasekharan (1970). In another set of experiment, same concentrations of culture filtrate were used to assay the toxin effect on excised guava twigs for symptom development. The excised guava twigs were dipped in large test tubes (2.5 × 22cm) containing toxin solution @ one twig per tube. Suitable control was maintained in both cases. The twigs showing wilting symptom (Rai and Strobel, 1969) and time required for initiation of wilting was recorded. The plants were transferred to 100 ml slightly saline sterilized distilled water in 150 ml conical flasks. Observation for the appearance of symptoms was made after 48 hrs of transfer. The expression of symptoms, if any, were noted as described under Table 4.

RESULTS AND DISCUSSION

The results presented in Table 1, 2 and 3 indicate

Table 1. Effect of toxic metabolites produced by *P. psidii* in synthetic media on rice seed vigour

Dilutions of toxins	Seed germination (%)			Mean radicle length (cm)			Mean plumule (cm)			Mean vigour index		
	CDB	MFB	RSB	CDB	MFB	RSB	CDB	MFB	RSB	CDB	MFB	RSB
N	78.9	78.0	73.0	3.7	3.2	0.7	2.5	2.1	0.5	650.0	468.0	102.0
N/1	80.9	80.0	80.5	5.1	3.4	1.3	3.1	2.9	0.9	802.1	505.6	361.3
N/2	86.6	84.0	83.0	5.5	3.6	1.9	3.4	3.3	1.4	990.5	607.2	407.5
N/3	89.9	87.0	85.0	5.8	3.9	2.0	4.7	3.4	2.8	1082.7	738.7	610.4
N/4	91.0	88.5	89.9	6.0	4.4	3.3	4.9	4.3	3.8	1092.7	888.4	757.4
Uninoculated media	92.7	92.7	92.6	6.1	6.2	6.1	5.0	5.0	5.0	1119.3	1119.1	1119.6
Distilled water (control)	93.9			6.5			5.4			1190.5		

Table 2. Effect of toxic metabolites produced by *C. gloesporioides* in synthetic media on rice seed vigour

Dilutions of toxins	Seed germination (%)			Mean radicle length (cm)			Mean plumule (cm)			Mean vigour index		
	CDB	MFB	RSB	CDB	MFB	RSB	CDB	MFB	RSB	CDB	MFB	RSB
N	75.0	00.0	00.0	2.0	0.0	0.0	1.6	0.0	0.0	306.0	000.0	000.0
N/1	78.8	70.0	00.0	3.5	1.4	0.0	2.5	1.3	0.0	456.0	248.3	000.0
N/2	83.3	81.2	56.5	4.2	2.7	0.7	3.0	2.5	0.6	607.5	361.9	114.7
N/3	85.0	85.0	76.5	5.7	3.7	1.6	4.0	3.7	1.6	821.7	575.1	246.2
N/4	92.0	87.5	83.7	6.0	4.7	2.3	4.5	4.7	2.2	1000.6	890.6	455.0
Uninoculated media	92.7	91.7	90.6	6.1	6.2	6.1	5.0	5.0	5.0	1119.3	1119.1	1119.6
Distilled water (control)	93.9			6.5			5.4			1190.5		

Table 3. Effect of toxic metabolites produced by *B. theobromae* in synthetic media on rice seed vigour

Dilutions of toxins	Seed germination (%)			Mean radicle length (cm)			Mean plumule (cm)			Mean vigour index		
	CDB	MFB	RSB	CDB	MFB	RSB	CDB	MFB	RSB	CDB	MFB	RSB
N	79.5	00.0	00.0	2.8	0.0	0.0	2.3	0.0	0.0	462.2	000.0	000.0
N/1	80.8	75.0	00.0	3.3	1.2	0.0	3.0	0.7	0.0	588.0	340.0	000.0
N/2	83.0	77.6	72.5	4.8	2.4	0.6	3.3	1.8	0.7	776.9	407.6	233.6
N/3	85.0	82.9	76.2	5.3	3.7	1.6	4.2	2.2	1.3	855.5	547.5	376.7
N/4	88.9	85.0	81.2	6.0	4.8	3.5	4.8	4.5	3.5	1064.8	923.0	722.3
Uninoculated media	92.7	92.7	92.6	6.1	6.2	6.1	5.0	5.0	5.0	1119.3	1119.1	1119.6
Distilled water (control)	93.9			6.5			5.4			1190.5		

*Each insertion is an average of 100 seed.

Comparison of significant effects	**SEm(±)**	**CD (P=0.01)**
Dilution of toxin	0.05	0.1
Meida	0.03	0.1
Pathogens	0.03	0.1
Dilution of toxins x Media	0.07	0.2
Dilution of toxin x pathogens	0.07	0.2
Media x pathogens	0.05	0.1
Dilutions of toxin x media x pathogens	0.13	0.3

Table 4. Effect of toxic metabolites produced by some foliar pathogens of guava in different synthetic media on excised guava twigs

Dilutions of	*P. psidii*			*C. gloeosporioides*			*B.theobromae*		
toxin(s)	C	F	R	C	F	R	C	F	R
N	++	+++	+++	–	+++	+++	–	+++	+++
N/1	+	+++	+++	–	+++	+++	–	+++	+++
N/2	–	++	+++	–	+++	+++	–	+++	+++
N/3	–	++	+++	–	++	++	–	++	++
N/4	–	+	+	–	+	+	–	+	+
Uninoculated media	–	–	—	–	–	–	–	–	–
Distilled water (Control)	–	–	–	–	–	–	–	–	–

– ⇒ No wilting

+++ ⇒ Severe wilting of apical meristem along with subsequent 2 to 3 tender leaves followed by chlorosis of leaf tips and leaf margins. In some cases light chlorotic zones were also noted in the interveinal spaces. This was subsequently followed by necrosis of leaf tips and leaf margins.

++ ⇒ Moderate wilting of apical meristem followed by first and second tender leaves or apical meristem. Yellowing of leaf tips and leaf margins in first and second tender leaves, no interveinal chlorotic and necrotic spot.

+ ⇒ Slightly wilting only of apical maristem (unferling leaves). no chlorosis was at any stage of symptoms development.

that all the pathogens produced the toxin(s) in all three synthetic media, showed adverse effect on germination of rice seed and subsequently on radicle and plumule length and vigour index. The maximum inhibition in seed germination, radical and plumule length and vigour index of rice seed was recorded in case of *C. gloeosporioides* followed by *B. theobromae* and *P. psidii* in RSB medium at N and N/1 toxin dilution than other dilutions. The effect of toxin on rice seed vigour also varied with respect to

dilution of toxin, pathogen and media used. On excised guava twigs, intense wilting was observed in toxin produced by *C. gloeosporioides, B. theobromae* and least in toxin produced by *P. psidii* both in RSB and MFB solutions at all dilutions except toxin dilution of 1 : 4. In CDB medium, none of the pathogen produced any wilt symptom irrespective of except *P. psidii* at N and N/1 concentrations. Extent of damage in excised guava twigs also varied similarly as earlier results.

From the over all perusal of the results it was found that among the three media used, RSB stimulated more toxin production as compared to MFB and CDB in supporting toxin production *in vitro.* Among the three pathogens *C. gloeosporioides* was the most potent toxin producer than *B. theobromae* and *P. psidii.*

Similar observation was made by Naik (1989) in *C. capsici* and some other seed borne fungi of rice. On the other hand, some workers like Liu and Yuan (2002) found CDB to be more suitable for production of toxin for *C. capsici* and *C. gloesporioides.*

The role of different cultural media in the production of toxic metabolites by *Aspergillus niger in vitro* have been elaborated. Other workers like Pringle and Scheffer, (1964) had categorically described the nutritional requirement of fungi in the production of toxic metabolites. It has been shown that the production of fusaric acid by *F. lycopersici, in vitro* varied considerably with the composition of the medium and that virulent and avirulent strains of pathogen reacted differently on changes in the medium composition (Egli, 1969).

The role of toxic metabolites in the deterioration of seed, quality and retardation in their subsequent germinability due to interference of toxic metabolites secreted by plant pathogenic fungi has been documented (Christensen and Kaufmann, 1965). Subsequently, Vidhyasekaran *et al.* (1970) have conclusively proved the interference of toxic metabolites produced by seed borne fungi like *F. moniliforme* in the deterioration of seed embryo and their subsequent failure to produce germtube. Considerable variation in the seed germination and seedling vigour may be due to variability in the fungi with regard to their toxin production and concentration (Niranjana and Sekhar Shetty, 1998 and Mohanraj *et. al.,* 2002). In the present experiment, the freshly cut healthy guava shoots were used to study the role of toxic metabolites in the production of disease symptoms and their similarities that are produced in host plant under natural condition. The present study clearly indicated that the toxic metabolites enter into host cells through injury of the tissues and cell membrane and were liable to cause damages to the host by this toxic principle (Mehan and Morphy, 1947).

The nature of symptoms produced in the present study by the toxic metabolites secreted by three pathogens in excised guava twigs. However, this may be concluded that the pathogen produced toxins of non-specific in nature. The result of the present experiment at level for *C. gloeosporioides* clearly established the positive role(s) of the toxic metabolites in the production of some parts of disease symptoms as under natural condition (Husain *et al.,* 1993) and the metabolites secreted by *C. capsici* contain more than one toxic principle (Narain and Das, 1970) and the metabolites produced by this pathogen is non-host specific in native.

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