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Physiology and effect of toxin produced by the *Colletotrichum capsici* Causing anthracnose of chilli (*Capsicum annuum* L.)

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Abstract
Colletotrichum capsici causing chilli anthracnose is one of the major economic constraints to chilli production in tropical and subtropical regions. The culture filtrate produced by the pathogen was tested for non-specificity under *in vitro*. The toxic metabolites produced phytotoxic symptoms in treated chilli fruits and leaves. The non-specific toxic metabolites in the culture filtrate reduced the seed germination, root length and shoot length of chilli, rice, cumbu, black gram, gingelly, tomato and brinjal. The total phenols, activity of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase increased in the pathogens inoculated riped and green chilli fruits compared to the corresponding healthy fruits. Total phenols and the activities of the enzymes was maximum at 3 days after inoculation and thereafter declined drastically in ripe chilli fruits where as in green chilli fruits, the activities of the enzymes reduced slowly. In general green chilli fruits showed higher accumulation of total phenols and also higher activities of enzymes than riped chilli fruits.

Keywords: anthracnose, seed germination, effect of toxin and enzyme activity

1. Introduction
Chilli (*Capsicum annuum* L.) belonging to the family solanaceae is of the important spice cum vegetable crop in India. The crop is grown over an area of 7.69 lakh hectares with a production of 12.39 lakh tonnes [1]. India is the largest consumer and exporter of chilli in the international market and exports dry chilli, chilli powder and olioresins to over 90 countries [2]. The major constraint to chilli production in India is fruit rot diseases, caused by *Colletotrichum capsici* (Syd.) Butler and Bisby. The yield losses of staggering dimension have been reported in various countries including India. *Colletotrichum capsici* is the most destructive disease of chilli, which cause pre and post emergence damping off, leaf spots, premature fruit drop, mummification of unripe green fruits and fruit rot, which contribute 50-100 % loss in India [3]. The chilli seed germination was affected by the toxin produced by *C. capsici* [4]. The microorganisms are pathogenic only if they are toxigenic. A vast array of toxins, host specific and non-specific are produced by plant pathogens and their role in symptom production has also been established [5]. Production of toxin *in vitro* by *C. capsici* has been reported [6]. With this background, in the present study, effects of toxin produced by *C. capsici* and to identify the non-specificity on different seeds were taken.

2. Material and Methods
2.1 Isolation of pathogen
The chilly plant exhibiting characteristic symptoms of anthracnose were used for isolation of *C. capsaici*. The pathogen was isolated through tissue segment method using PDA medium [7] and pure culture was obtained by single spore isolation technique [8]. The identity of pathogen was confirmed through morphological and cultural examination of ten days old test pathogen using MZ-16 Leica image analyser.

2.2 Production of the toxin
Ten mm of seven day old PDA culture disc of pathogen was inoculated into 100 ml sterilized PDA medium contained 500 ml saline dextrose bottle and incubated at room temperature (28 $\pm$ 2^{o}C). The mycelial mat was removed by filtering through Whatman No. 1 filter paper after 15 days. The culture filtrate was centrifuged at 200 g for 20 min and used for further assay and extraction.

2.3 Effect of culture filtrate

PDA broth was used for toxin production by *C. capsici*. The medium was dispended at the rate of 50 ml in 250 ml Erlenmeyer flask and sterilized by autoclaving at 1.04 kg cm^{-2} for 20 min. Flasks were inoculated with the fungus and incubated at 27°C for 15 days. After the incubation period, the culture filtrate was separated from the mycelial mat by filtration. The culture filtrates were used as the source of toxin [9]. The filtrate was centrifuged at 2000 g for 20 min. Further extraction was done as per the procedure [10]. The residue (crude toxin) was taken in 2 ml of distilled methanol, after evaporating the supernatant at room temperature (27 ± 1°C).

2.4 Effect of culture filtrate

The toxicity of the culture filtrate against seed germination and seedling growth of chilli, rice, maize, sorghum, cumbu, blackgram, greengram, gingelly, cluster bean, tomato and brinjal, were tested under laboratory condition as described [11]. Seeds of chilli, rice, maize, sorghum, cumbu, blackgram, greengram, gingelly, cluster bean, tomato and brinjal, were surface sterilized with 0.1% mercuric chloride solution for 30 sec washed repeated by using sterile distilled water and soaked in 25 ml of the culture filtrate for 24 h except black gram (5 h). Twenty five seeds were placed at equidistance on the sterilized filter paper kept inside the sterile Petri dishes and wet with 10 ml of sterile distilled water. Three replications were maintained. The seeds soaked in sterile distilled water served as control. The germination was recorded after 5 day and the per cent germination was calculated. The length of shoot and root of the seedlings was also recorded after 5 days.

2.5 Physiology of chilli fruits inoculated with C. capsici

The riped and green chilli fruits were surface sterilized with 0.1% mercuric chloride for 20 sec, washed with sterile distilled water and inoculated with *C. capsici*. The fruits treated with sterile distilled water served as control. The fruits were collected at various time intervals (1, 2, 3, 4 and 5 days after inoculation) and used for biochemical analysis. The total phenols, phenylalanine ammonia layse, peroxidase and polyphenol oxidase present in the fruits were estimated by the methods [12-15] respectively.

3. Results and Discussion

The pathogen was isolated from chilli fruits showing the typical symptoms of fruit rot, purified and cultures of these isolates were maintained on Potato Dextrose Agar (PDA) slants. The symptoms of the disease appeared as small circular spots appeared on the skin of the fruit, later it spread in the direction of the long axis of the fruit. The spots were sunken and light grey coloured with black margin. The spots enlarged into larger lesions and on the surface of the lesions acervuli, the fruiting body of the fungus appeared as minute black dots. The morphological characters of all the isolates of the chilli fruits rot pathogen were pertinent to *C. capsici* as per the original descriptions [16]. The fruiting bodies *viz*., acervuli were produced on the infected area. The infection spread to the entire fruit resulting in drying of the fruits. Seeds of different crop plants were tested for the effect of the culture filtrate, highest inhibition of seed germination was observed in chilli when compared to other crops. Culture filtrate treated chillies recorded the lowest seed germination of 26.67 % as against 94.67 % in control with 71.83 % inhibition over control (Table 1). This was followed by tomato (34.67) as against 93.33 % seed germination in control with 62.85 % inhibition over control, brinjal (37.33) as against 94.67 % seed germination with 60.57 % inhibition over control. The inhibition of seed germination of green gram, sorghum, cluster bean, maize, rice, black gram, gingelly and cumbu varied from 2.82 to 37.84 % over control. The reduction of shoot length was highest in chilli (76.92 %) followed by black gram (68.63 %) and least inhibition of 4.42 % shoot length were recorded in cluster bean (Table 2). The culture filtrate caused the highest reduction of root length in chilli (76.46 %) followed by black gram (48.66 %) and least inhibition of 12.96 % root length was recorded in sorghum (Table 3). In present study, *C. capsici* produced a toxic metabolite in the culture filtrate and it was inhibitory to the seed germination, shoot and root elongation of seedlings of chilli. The toxin also affected the seed germination, shoot and root elongation of rice, maize, cumbu, sorghum, blackgram, greengram, gingelly, cluster bean, tomato and brinjal. Similar type of observation was made by [17] who reported the inhibitory effect of toxic metabolite of *C. capsici* in chilli seeds and seedlings. The toxic metabolite of *C. capsici* inhibited the radical growth of chilli, greengram, pea and cowpea cultivars [5]. Similarly, the toxin of *C. capsici* inhibited the seed germination of chilli, Mungbean and groundnut [4]. The crude toxin of *C. capsici* completely inhibited the seed germination of chilli [18].

Table 1: Effect of culture filtrate of *C. capsici* on seed germination

seeds	Treated	Untreated	% reduction over control
Chilli	26.67	94.67	71.83
	(31.04)	(76.83)	
Rice	82.67	98.67	16.22
	(65.43)	(82.82)	
Maize	85.33	97.33	12.33
	(67.53)	(80.64)	
Cumbu	61.33	98.67	37.84
	(51.56)	(82.82)	
Sorghum	93.33	100.00	6.67
	(75.20)	(85.00)	
Black gram	74.67	96.00	22.22
	(59.85)	(78.47)	
Green gram	92.00	94.67	2.82
	(73.92)	(76.83)	
Gingelly	64.00	97.33	34.24
	(53.20)	(80.64)	
Cluster bean	90.67	98.67	8.11
	(72.29)	(82.82)	
Tomato	34.67	93.33	62.85
	(36.06)	(75.20)	
Brinjal	37.33	94.67	60.57
	(37.66)	(76.83)	
CD (P=0.05%)	4.58	5.18	-

* Mean of three replications
Data in parentheses are arc sine transformed values

Table 2: Effect of culture filtrate of *C. capsici* on shoot length

Seeds	Treated	Untreated	% reduction over control
Chilli	1.23	5.33	76.92
Rice	5.17	7.77	33.46
Maize	4.93	6.30	21.75
Cumbu	4.47	5.57	19.75
Sorghum	5.17	7.57	31.70
Black gram	1.17	3.73	68.63
Green gram	3.33	6.43	48.21
Gingelly	2.57	4.30	40.23
Cluster bean	4.97	5.20	4.42
Tomato	3.13	5.13	38.99
Brinjal	1.73	4.27	59.48
CD (P=0.05%)	0.11	0.18	-

* Mean of three replications

Table 3: Effect of culture filtrate of *C. capsici* on root length

Seeds	Treated	Untreated	% reduction over control
Chilli	1.17	4.97	76.46
Rice	4.93	6.53	24.50
Maize	4.97	7.73	35.71
Cumbu	4.27	5.27	18.98
Sorghum	4.03	4.63	12.96
Black gram	1.73	3.37	48.66
Green gram	1.87	3.57	47.62
Gingelly	2.97	4.36	32.04
Cluster bean	4.30	6.37	32.50
Tomato	3.13	4.87	35.73
Brinjal	3.17	4.37	27.46
CD (P=0.05%)	0.14	0.19	-

* Mean of three replications

Total phenols increased both in riped and green chilli fruits in response to inoculation with *Colletotrichum capsici* as compared to uninoculated ones. In green chilli fruit also the highest level of total phenols of 45.0 mg /100 g was found on 3rd day after inoculation (Fig. 1). Phenolics has performed inhibitory substance have been reported in several host-pathogen interactions [19]. Green chilli fruits had higher level of total phenols than riped chilli fruits. The decrease in phenolic compounds in chilli as the fruit ripens has been reported [20]. High level of phenolics in green chilli fruits may be one of the reasons for the inability of *C. capsici* to infect green chilli fruits. The phenolic content decreased three days after inoculation and this decrease is very much pronounced in riped chilli fruits than in green chilli fruits. The decrease in phenolic content coincides with necrosis of cells in the inoculated tissues. In general, PAL, PO and PPO enzymes activities were increased in riped chilli fruit from 1st day after inoculation and reached maximum on 3rd day after inoculation (Fig. 2). This study indicated that the green chilli fruit had more PO activity than riped chilli fruits. The activity of the enzymes phenyalanine ammonia lyase (PAL), peroxidase (PO) and polyphenol oxidase increased in inoculated fruits compared to the corresponding healthy fruits. The activity of these enzymes increased in the initial stages of the disease and decreased as the disease progressed coinciding with necrosis of tissues. PAL is the first enzyme of the phenylpropanoid pathway which leads to the synthesis of phenolics. PAL catalyzes the conversion of phenylalanine to transcinnamic acid in the first step of phenylpropanoid pathway from which other phenolics, phytoalexins and lignin are synthesized [21]. In the present study, though PAL activity increased both in riped and green chilli fruits in response to inoculation with fruit rot pathogens, the increase was much more in green chilli fruits than in riped chilli fruits. Higher increase in PAL activity in inoculated. The polyphenol oxidase and peroxidase might function as an alternate electron transport chain and serve as terminal oxidase in infected plant tissue [23]. The factors that trigger these enzymes are subject of study for several works during the past four decades [24, 25]. Higher activities of PAL, PO and PPO in green chilli fruits than in riped chilli fruits and rapid increase in the activities of these enzymes in green chilli fruits than in riped chilli fruits in response to inoculation with *C. capsici* suggest the probable role of these enzymes in imparting resistance in green chilli fruits to fit it rot pathogens.

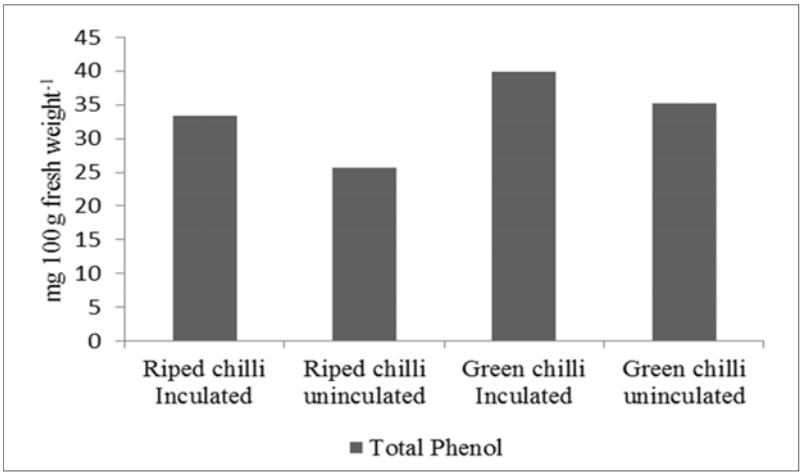

Fig 1: Changes in total phenol activities in chilli inoculated with *C. capsici*

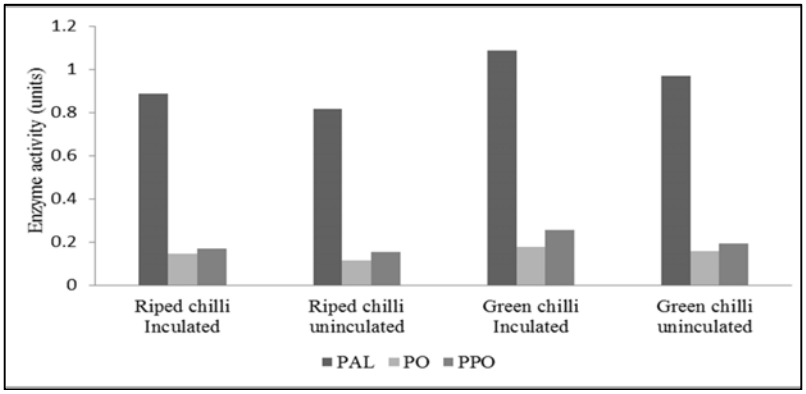

Fig 2: Changes in PAL, PO and PPO activities in chilli inoculated with *C. capsici*

4. Conclusion

The toxin produced by *Colletotrichum capsici* reduced the chilli seed germination and showed non-host specificity against various crop plants. The application of toxin on chilli fruits were showed the increased synthesis of various enzyme activities, thereafter reduced level of resistance was observed due to toxin effect. The control of seed borne nature of toxin producing fungus *Colletotrichum capsici* by various strategies could be useful in reducing crop loss.

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