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Toxic Metabolite Production by *Colletotrichum gloesporioides* Causing Blight of Coriander

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Abstract : The pathogen *Colletotrichum gloeosporioides* produced non specific toxic metabolites in culture filtrate. Drooping and wilting were the striking symptoms observed in most of the plant species tested in culture filtrate but the time taken for expression symptoms varied from plant to plant. Maximum amount of toxin was produced on 16th day of incubation. Culture filtrate inhibited the seed germination of coriander, tomato and radish drastically. The toxin proved to be thermostable and retained its toxicity even after autoclaving at 1.1 kg/cm^2 pressure for 15 minutes, while, by diluting with water, its toxicity was reduced to a great extent. The dilutions of the culture filtrate and the time taken for symptom expression were positively related.

Introduction

It is a well established fact that in many plant disease, toxins produced either by pathogen or by host or by interaction of both play an important role in pathogenesis (Wheeler and Luke, 1963; Pringle and Scheffer, 1964; Sadasivan, 1969). The same is true in certain species of *Colletotrichum*, which cause various disorders in plants (Goodman, 1958; Sharma and Sharma, 1969; Narain and Das, 1970; Santhakumari, 1980). The coriander blight caused by *C.gloecosporioides* (Penz.) Penz. and Sac., is more prevalent in Karnataka and causes much damage to the crop (Naik *et al.*, 1988). The pathogen produces a characteristic necrotic lesions surrounded by a diffused yellow halo, which is very indicative of role of toxic substance secreted by the pathogen in the genesis of such yellow halo region. Therefore, such studies would disclose the implications in the physiology of tissue development.

Material and Methods

A virulent culture of *C.gloeosporioides* isolated from coriander plant showing typical blight symptom was used in these studies. The culture was maintained on 2 per cent potato dextrose agar medium. To ascertain growth period at which the fungus produces maximum toxic metabolite in Richards' broth (as basal medium), *C.gloeosporioides* culture was harvested at an interval of 2 days, starting from 2nd day of incubation upto 30th day. The bioassay of toxic metabolite was made on coriander plant cuttings.

To study the specificity of toxic metabolite, coriander, betel vine, tomato, soyabean, potato, alfalfa, bittergourd, pea and groundnut plant cut-

tings were used following the procedures of Rai and Strobel (1969) and Sharma and Sharma (1969).

The plant cuttings of same size and age were placed in test tubes containing equal volume of culture filtrate obtained from 16 day old *C. gloeosporioides culture*. Control was run simultaneously with sterile water. The time required for the symptom development and the nature of symptoms were recorded. Similarly, bioassay of toxic metabolite was made on seed germination following the methds described by Ludwig (1957) and Anahosur (1976). Coriander, redgram, bengalgram, mustard, radish, sorghum, wheat and tomato seeds were soaked in 16 day old culture filtrate separately for 12 hours and later placed in petridishes lined with sterilized filter papers moistened with the culture filtrate. Control was run simultaneously with uninoculated broth and sterile water. The germination was counted five days after incubation and the reduction in per cent germination over control was calculated.

To study the effect of heat on culture filtrate, an aliquot of 5 ml was flamed at 50° and 70° C to get warm. Another set was heated to 100°C for 10 minutes and the fourth one was autoclaved at 1.1 kg/cm^2 pressure for 15 minutes. All these treatments were assayed on host plants for development of symptoms.

To study the effect of dilution of culture filtrate, the young host cuttings taken from 20 day old plants were treated with 10 ml of test solution. The stock culture filtrate served as control. Each treatment was replicated thrice. Plants were observed for symptom development at regular intervals.

Results and Discussion

The plant cuttings dipped in the culture filtrate obtained on second day remained apparently healthy, which indicates that the *C. gloeosporioides* might not have produced any toxic metabolite in that short time or the amount of toxic metabolite produced might be insufficinet to express symptoms on plant cuttings. Drooping of twigs was evident in culture filtrate obtained from 4th day onwards, but the time taken for expression varied from 3 to 48 hours (Table 1). As the age of the culture increased, the time required to express symptoms decreased, indicating increased amount of toxic metabolite in the culture filtrate. But from the 16th day onwards, the time remained constant. This clearly indicates that the maximum toxic metabolite of the fungus could be harvested on 16th day after incubation.

All the plant species dipped in the culture filtrate showed disease syndrome like drooping and some kind of discolouration, but the time taken to express such symptoms varied from plant to plant. Plant cuttings of bittergourd, pea and groundnut took more time for symptom expression than other plant cuttings tested (Table 2). The study clearly indicates that *C. gloeosporioides* produces non-specific toxic metabolite.Sharma and Sharma (1969) noticed similar trend in an isolate of *C. gloeosporioides*, a causal agent of citrus die-back. The toxic metabolite also played a significant role in reducing the seed germination of various crop plants. Maximum reduction of seed germination was in coriander, followed by tomato, radish and bengal gram and the least was in wheat (Table 3). Vidyasekharan *et al.* (1970) observed the reduction of seed germination in paddy due to toxin produced by seed-borne fungi. The sorghum seed germination was not inhibited. It clearly indicates that the seeds of vegetables are most susceptible to toxic metabolite of the fungus followed by pulses and oil seed crops.

The culture filtrate even after boiling at 100°C for 10 minutes and autoclaving at 1.1 kg/cm^2 pressure for 15 minutes did not loose its toxic effect and resulted in drooping, wilting and

Table 1. Production of toxic metabolite by *Colletotrichum gloeosporioides* in Richards' solution at different incubation periods and its effect.

Days after seeding	Time taken for symptom expression on coriander plant cuttings (in hr)	Symptoms
2	48	Cuttings remained healthy
4	48	Drooping
6	30	Drooping and wilting
8	18	Drooping, browning of leaves and wilting
10	10	Drooping, browning, wilting and drying
12	6	Drooping, browning, wilting and drying
14	5	Drooping, curling, complete wilting and brittling
16	3	Drooping, severe wilting, curling, browning and brittling of leaves
18	3	Drooping of twig, browning, curling and brittling
20	3	Drooping, wilting, browning, curling and drying
22	3	Wilting, browning of leaves and drying
24	3	Drooping, browning, wilting and curling
26	4	Drooping, browning, curling and wilting
28	4	Browning, drooping and curling
30	3	Drooping, browning, marginal curling and severe wilting

Table 2. Effect of 16 day old culture filtrate of *Colletotrichum gloeosporioides* on plant cuttings of different species

Plant species	Time taken for expression of symptoms (in hr)	Symptoms
Tomato	2	Drooping, browning, marginal necrosis, curling of leaves and wilting
Coriander	3	Drooping, curling of leaves and wilting
Betel vine	3	Drooping, severe wilting and curling of leaf margin
Potato	4	Drooping, marginal necrosis, curling, brittling of leaves and wilting
Alfalfa	4	Drooping, curling of leaves and wilting
Soyabean	4	Drooping, curling of leaves and wilting
Bittergourd	10	Drooping of vine
Pea	12	Drooping only
Groundnut	48	Drooping only

Table 3. Effect of 16 day old culture filtrate of *Colletotrichum gloeosporioides* on seed germination of different plant species

Plant species	Reduction in seed germination (in per cent)
Coriander	44
Tomato	38
Radish	32
Bengalgram	17
Redgram	14
Mustard	12
Wheat	2
Sorghum	0

browning symptoms on host tissues. Thus, the toxic principle proved to be thermostable. The thermostable toxins were also observed in *Helminthosporium victoriae* (Litzenberger, 1949) and *C. capsici* (Nair, 1972). Thus, the result clearly indicates the least possibility of inactivation of toxin of *C. gloeosporioides* in host tissues in nature due to atmospheric temperature.

In the stock culture filtrate and 1:5, 1:10 and 1:100 dilutions, the common symptoms like drooping and wilting were noticed. In addition to drooping and wilting, browning, curling and brittling were seen in the plant cuttings kept in stock solution of culture filtrate and these symptoms were expressed within 3 hours of incubation (Table 4). The dilutions of culture filtrate and the time taken for the symptom development were positively related and is in agreement with the results obtained by Balakrishna (1975), in respect of toxic metabolite produced by *Septoria lycopersici.*

Table 4. Effect of dilution of culture filtrate of *Colletotrichum gloeosporioides* on coriander plant cuttings

Dulutions	Time taken for symptom expression (in hr)	Symptoms
1 : 5	8	Drooping and wilting
1 : 10	15	Drooping and wilting
1 : 100	36	Drooping and wilting
1 : 1000	36	Cuttings remained healthy
Stock culture filtrate	3	Drooping, wilting, curling, browning and brittling

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