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# Toxin studies of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., causal agent of Anthracnose of pomegranate

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Pomegranate is an important fruit crop of India and subtropical countries of the world. *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. is causal agent of anthracnose of pomegranate which affects yield and quality of fruits. In the present study, toxin production by *C. gloeosporioides* in *vitro* was studied by sorghum seed germination, root and shoot elongation and tomato seedlings growth inhibition in culture filtrate. Maximum inhibition of sorghum seed germination (60.30%) and also roots (74.10%) and shoot (56.80%) length was noticed in 72 h. of 20 days old culture filtrate. Increase in the days of culture filtrate reduced seed germination and affected shoot and root length. Further tomato seedlings of same age and size were kept in 2 to 20 days old culture filtrate and complete wilting of seedlings was observed within 72 h. Two days old culture filtrate showed complete wilting after 96 h. Symptom expression increased with increase in days of incubation from 2 to 20 days. Untreated sterile water control showed no symptoms at all. The present findings clearly indicated that, active metabolite released by *C. gloeosporioides* in culture was toxic to seed germination of sorghum and growth of tomato seedlings.

**Key words :** *Colletotrichum glaeosporioides*, toxin, anthracnose, pomegranate

## INTRODUCTION:

Pomegranate (*Punica granatum* L.) is commercially an important fruit crop belongs to the family Punicaceae. Pomegranate suffers from ten economically important diseases, among them fruit rot, bacterial spot/blight, wilt and anthracnose are severe and cause significant losses in recent years. Anthracnose caused by *Colletotrichum gloeosporioides* is the most severe disease in Karnataka which causes spotting and rotting of pomegranate fruit leading to decrease in quality and market price. Screening of genotypes by toxin sensitivity test is the easiest, time, space and energy saving method. Hence, in the present study, toxin production by *C. gloeosporioides* in *vitro* has been studied by sorghum seed germination, root and shoot elongation and tomato seedlings growth inhibition in culture filtrate of Potato Dextrose broth.

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## MATERIALS AND METHODS

This experiment was conducted as per the procedure followed by Anahousr (1976). One hundred healthy sorghum seeds were surface sterilized in 1% sodium hypochlorite solution and washed with sterile distilled water to remove the traces of sodium hypochlorite. The sorghum seeds were soaked in culture filtrates for 30 minutes. They were spread on moistened germination paper. Equal numbers of healthy seeds were soaked in the sterile distilled water and Potato dextrose broth, which served as control. Observations on germination of sorghum seeds were recorded after 7 days. Per cent inhibition of seed germination was calculated by the formula given by Vincent (1947). Further root and shoot lengths were recorded in each treatment and seedling vigour index was calculated.

Seedling vigour = (Shoot length + Root length) × Germination percentage

***Effect of culture filtrate on tomato seedlings***

This experiment was carried out to know production of toxic metabolites by pathogen. The culture filtrates of *Colletotrichum gloeosporioides* obtained at an interval of two days from 2 to 20 days old culture was subjected to find effect of this culture filtrate on tomato seedlings. Then 25 days old seedlings of same size were selected, and placed in test tubes containing 15 ml of different days old culture filtrates. Each treatment was replicated three times and control was maintained with sterile distilled water and un-inoculated Potato dextrose broth. Effects of culture filtrate on tomato seedlings were recorded at 12 h and 24 hr, 36 hr and 48 h after incubation.Observations on drooping of leaves, curling, marginal necrosis and wilting symptoms were also recorded.

## RESULTS AND DISCUSSION

A preliminary indication of toxin production by any fungus *in vitro* has been provided by a number of bioassay methods of plant cutting (Sharma and Sharma, 1969; Naik *et al.*, 1991), seed germination bioassay , root and shoot elongation bioassay (Anahosur, 1976, Venkataravanappa, 2002) and spore germination inhibition method (Sharma and Sharma, 1969).

Effect of culture filtrates were studied on seed germination, shoot and root length of sorghum seedlings. Maximum inhibition of sorghum seed germination and also root and shoot length was recorded.

The maximum per cent inhibition of seed germi-

**Table 1:** Effect of age of culture filtrate of *Colletotrichum gloeosporioides* on percent inhibition of seed germination, shoot and root length of sorghum

| Treatments (days) | Per cent inhibition | | | Vigour index |
|---|---|---|---|---|
| | Seed germination | Shoot length | Root length | |
| 2 | 20.00 (26.55)* | 11.00 (19.3) | 11.40 (19.76) | 2048.00 |
| 4 | 23.70 (29.09) | 18.00 (25.10) | 17.90 (25.03) | 1776.75 |
| 6 | 33.00 (35.02) | 25.00 (30.00) | 34.30 (35.86) | 1293.39 |
| 8 | 39.00 (38.63) | 27.70 (31.70) | 37.40 (37.66) | 1193.77 |
| 10 | 41.00 (39.77) | 34.10 (35.70) | 39.10 (38.66) | 1064.30 |
| 12 | 42.30 (40.57) | 39.10 (38.80) | 43.30 (41.13) | 957.60 |
| 14 | 50.70 (45.36) | 43.50 (41.30) | 50.20 (43.56) | 751.20 |
| 16 | 52.30 (46.31) | 48.30 (44.00) | 60.20 (49.33) | 623.30 |
| 18 | 60.30 (50.94) | 48.40 (44.20) | 61.00 (50.96) | 468.30 |
| 20 | 60.30 (5.94) | 56.80 (48.90) | 74.10 (59.36) | 379.80 |
| Control (sterile distilled water) | 0.00 | 0.00 | 0.00 | 2700.00 |
| S.Em. ± | 0.93 | 0.43 | 1.05 | |
| CD @ 1% | 2.66 | 1.23 | 3.00 | |

*Arcsine transformed values

nation was recorded in both 18 and 20 days culture filtrate (60.30%) which differed significantly with other treatments. Least per cent seed germination inhibition was recorded in 2 days (20.00%) culture filtrate which was at par with 4 days (23.70%) culture filtrate. Maximum inhibition of sorghum shoot length (56.80%) and root length (74.10%) were recorded in 20 days old culture filtrate followed by days 18 and 16 days and were at par with each other. Least per cent shoot length and root length inhibition was recorded in 2 days culture filtrate (11.00 and 11.40%) Further, the seedling vigour index was calculated and presented in the Table 1. It was noticed that higher seedling vigour index was noticed in sterile distilled water (2700) followed by 2 days old culture

**Table 2:** Effect of age culture filtrate of *Colletotrichum gloeosporioides* on tomato seedlings up to 96 hours of incubation

| Treatments (days) | Incubation period (h) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 12 | 24 | 36 | 48 | 60 | 72 | 84 | 96 |
| 2 | NS | NS | D | CL | MN | PW | PW | CW |
| 4 | NS | NS | D | CL | MN | PW | PW | CW |
| 6 | NS | D | CL, | B | MN | PW | PW | CW |
| 8 | NS | D | D,CL | MN | PW | PW | CD | CW |
| 10 | NS | D | B, CL | B, CL | PW | PW | CD | CW |
| 12 | NS | D, MN | B, CL | B, CL | PW | PW | CW | - |
| 14 | NS | D,MN | B,CL | PW | PW | PW | CW | - |
| 16 | NS | D, MN, CL | B, | B, CL | PW | CD | CW | - |
| 18 | NS | D, MN, CL | B | B | PW | CD | CW | - |
| 20 | NS | D, MN, CL | B | PW | CD | CW | CW | - |
| Control (sterile distilled water ) | NS | NS | NS | NS | NS | NS | NS | NS |

D: Drooping; CD : Complete drooping; PW :Partial wilting; B : Browning; CL : Curling of leaves; MN : Marginal necrosis; CW: Complete wilting, BL: Brittling of leaves; NS : No symptoms

filtrate (2048). While, the least vigour index was noticed in 20 days old culture filtrate (379.80) followed by 18 days culture filtrare (468.30).

Tomato seedlings of same age and size were kept in culture filtrates of 2 to 20 days of *C. gloeosporioides.* The results on the effect of these culture filtrates on tomato seedling recorded at 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, and 96 h of incubation are presented in Table 2. No symptoms were found up to 12 h in all treatments. Among the different culture filtrates, 2 days and 6 days culture filtrates showed symptom after 24 h. Slight drooping of bottom leaves, marginal necrosis was the initial symptoms observed in all treatments at 24 h. The tomato seedlings showed partial (with in 60 – 72 h) and complete wilting (with in 72 – 96 hr). Whereas 20 days culture filtrate showed complete wilting within 72 h but 2 days old culture filtrate showed complete wilting after 96 h. Symptom expression increased with increase in days of incubation from 2 to 20 days. The results are in agreement with observations of Sharma and Sharma (1969) and Venkataravanappa (2002) who reported toxicity of culture filtrate of *Colletotrichum gloeosporioides* on sorghum seeds and tomato seedlings.

Increase in the days of culture filtrate the seed

germination, shoot and root length and tomato seedling growth inhibition decreased showing in linear manner.The present findings clearly indicated that, active metabolite released by the fungus in culture filtrate was toxic to seed germination of sorghum and growth of tomato seedlings. These findings are in agreement with Venkataravanappa (2002) who reported that *Colletotrichum gloeosporioides* produced nonspecific toxic metabolites in culture filtrate, which inhibited the seed germination of sorghum. Jayashankar *et al.* (1999) found that the *Colletotrichum gloeosporioides* produced phytotoxin *in vitro,* which inhibited the seed germination of lettuce and tobacco seeds.

## REFERENCES

Anahosur, K. H., 1976, Toxic effect of the culture filtrate of *Trochothecium roseum* on seed germination and growth of sorghum. *Indian Phytopathol.,* **29**: 278-280

Jayashankar, S., Gray, R. E., and Moon, P. A., 1999, Responses of embryogenic mango cultures and seedlings bioassays to a partially purified phytotoxin produced by mango leaf isolates of *Colletotrichum gloeosporioides* Penz. *In vitro. Cellular and Devp Biol. Pl.*, **35**: 475-479.

Naik, K. S., Hiremath, P. C. and Hegde, R. K., 1991, Toxic metabolite production by *Colletotrichum gloeosporioides* causing blight of coriander. *Karnataka J. Agric. Sci.,* **4**: 27-31

Sharma, M. C. and Sharma, B. C., 1969, Toxic metabolites production by *Colletotrichum gloeosporioides* causing citrus dieback in India. *Indian Phytopathol.,* **22**: 65-73.

Venkataravanappa, V., 2002, Studies on mango annthracnose disease caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. *M. Sc. (Agri.) Thesis*, Uni. Agric. Sci. Bangalore (India).

Vincent, J. M., 1947, Distribution of fungal hyphae in the presence of certain inhibitors. *Nature*, **159**: 850.