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TRICHODERMA SPECIES AS BIO-CONTROL AGENTS AGAINST ANTHRACNOSE OF POMEGRANATE (*PUNICA GRANATUM* L.) CAUSED BY *COLLETOTRICHUM GLOEOSPORIOIDES* PENZ.

M. Nagalakshmi Devamma[1], P. Suvarnalatha Devi[2], B.Jyothi[3], R.Ranjani[4], M. Rajaswi Devi[5]*
[1]Department of Botany,[4]Department of Virology, Sri Venkateswara University, [2]Department of Applied Microbiology, [5*]Department of Biochemistry, Sri Padmavathi Mahila Visvavidyalayam, [3]Department of Botany, S.P.W Degree & PG College. Tirupati-517502, Andhra Pradesh, India.

***Corresponding Author:** devi4.mandadi@gmail.com

Abstract

The importance of Pomegranate (*Punica granatum* L.) being economical, medicinal valued fruit and also rich in vitamins. It's an epitome of health, fertility and eternal life. However, Pomegranate is facing a threat through important diseases like anthracnose, caused by *Colletotrichum gloeosporioides* Penz. The threat is so severe that it affects the fruit at orchard and post-harvest stages as a result, yield and quality reduction along with marketability loss. The solution to this threat is through biological approach, which means the induction of the seven fungal isolates were evaluated by dual culture technique for their antagonistic activity against *Colletotrichum gloeosporioides* under invitro conditions is more eco-friendly than chemical approach. Among seven isolates the maximum inhibition of mycelial growth (73.50%) was noticed in *Trichoderma harzianum* (Madanapalle field isolate) which was followed by *Trichoderma viride* (Palamaner field isolate) (60.43%). Least inhibition was observed in *Trichoderma harzianum* (Punganur field isolate) (29.70%). The results indicated that the application of these fungi successfully reduces the anthracnose incidence and also increases the yield of the Pomegranate fruits.

Keywords: Pomegranate, Anthracnose, *Colletotrichum gloeosporioides*, biological control, *Trichoderma.*

Introduction

Pomegranate (*Punica granatum* L.) belongs to Family Punicaceae. It is a small tree, measuring less than 4 m when cultivated, although it can reach 7 m in length. Some trees may live longer than 100 years. The root is knotty, consistent and reddish, well developed and extremely absorbent in saline soils. The leaves measure about 2 to 9 cm in length and 1 to 3 cm in width. They are entire, smooth, opposed, with no stipule, sometimes

verticillate, hairless, oblong, deciduous and with short petioles. The flowers appear singly or in small clusters generally of 2-7 flowers, occasionally at the end of the branch but sometimes on the auxiliary buds. The fruit is a fleshy berry denominated balausta, thickskinned, complex, enclosed by the thallus, with various polyspermal cavities separated by tenuous membranous partitions (carpelar membranes). The interior is filled with many fleshy seeds, prismatic in shape, with pulpy testa and woody tegmen, very juicy. The ripe fruit is greenish yellow or brown with reddish areas which may occasionally occupy the whole surface of the fruit. The main diseases affecting pomegranate fruit are viz., Alternaria fruit rot (*Alternaria alternate*), Aspergillus fruit rot (*Aspergillus niger*) and gray mold (*Botrytis cinerea*). Fusarium wilt and Anthracnose (*Colletotrichum gloeosporioides).* Among these Anthracnose caused by (*Colletotrichum gloeosporioides)* which effects crop seriously. It is economically important pathogen on numerous fruit crops worldwide. Anthracnose is most severe disease which causes spotting and rot of pomegranate fruits leading to decrease in quality and market price.

Symptoms:

- Small, regular to irregular black spots on leaves, calyx region and fruits which turn later on as dark brown depressed spots. Infected leaves turn yellow and drop off.

Pomegranate fruits Infected with Anthracnose Disease

Infected leaves are Primary source of inoculum. Secondary source of inoculum is wind born conidia.The disease is severe during August-September when there is high humidity, and the temperature between 20-27ºC. In agriculture, there is an important need for alternate ecofriendly management to control plant diseases. Natural management of the disease through antagonists is an eco-friendly approach apart from better alternative in place of chemicals. Trichoderma sp. has been reported to be potential antagonists and these gained considerable success for the control of plant diseases [1, 2, and 3].

The application of bio-control agents is the key elements for sustainable agriculture. Therefore, the adoption of a sustainable agricultural practice, using strategies that are environmentally friendly, less dependent on agricultural chemicals is gaining worldwide recognition. In view of the above findings the present study was carried out by some of the beneficial bio agents collected from different farmer's fields and tested against the *Colletotrichum gloeosporioides* to determine their antagonistic potential in vitro.

Material and Methods:

Isolation and identification of the pathogen:

A total of seven fungal isolates were collected in the regions where the disease incidence is severe according to the information given by the local farmers during the survey. The infected fruit was cut into small bits and washed in running water. These bits were surface sterilized with 1 per cent mercuric chloride solution and then aseptically transferred to petri plates containing sterilized PDA medium. The plates were incubated at 27±1°C for three days. The fungal growth on fourth day, which arise through the infected tissue was taken by inoculation loop and transferred aseptically to the PDA slants. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture method under aseptic conditions [5]. The colonies of *Colletotrichum gloeosporioides* isolates on PDA were initially white and later became dull grey to dark grey. Mycelium was sparsely septate and dark brown in colour. conidia were hyaline, aseptate, cylindrical with rounded ends containing one or two oil globules,on reverse the colonies produced pink to orange colour with or without concentric rings.

Details of *C.gloeosporioides* Penz., collected from Chittoor district of Andhra Pradesh

S.No	Sample no.	Place of Collection	District
1.	MDP1	Madanapalle	Chittoor
2.	PTR2	Puttur	Chittoor
3.	PGR3	Punganur	Chittoor
4.	DMC4	Damalacheruvu	Chittoor
5.	PLR5	Palamaneru	Chittoor
6.	KPM6	Kuppam	Chittoor
7.	RSD7	Ramasamudram	Chittoor

Pure cultures of *Colletotrichum gloeosporioides*
Isolated from different Mandals of Chittoor district

Conidia of *Colletotrichum gloeosporioides* (45x)

Pathogenicity

Pathogenicity of different isolates of *C.gloeosporioides* was tested by artificial inoculation method using mature pomegranate fruits. The pathogen was reisolated from the infected fruits and it was found that to be the same as the original culture there by Koch's postulates were proved for all the seven isolates of *C.gloeosporioides.*

Disease Severity

The variation was observed among the isolates of *C.gloeosporioides* with regard to per cent disease incidence (PDI). Based on PDI, the isolates were classified as less virulent, moderately virulent and highly virulent.

Classification of *C.gloeosporioides* isolates based on percent disease incidence (PDI)

Virulence factors	Isolates
Highly virulent	DMC4
Moderately virulent	MDP1,PGR3,PLR5,RSD7
Less virulent	PTR2,KPM6

The maximum incidence of disease was recorded in isolate DMC4 (76.30%) followed by MDP1 (47.90%), PGR 3 (40.25%), PLR5 (37.65%), RSD7 (31.90%), and the least PDI was recorded in PTR2 (19.68%) and KPM6 (12.55%). There is a significant difference with respect to pathogenicity among all the isolates.

Isolation and identification of native potential antagonists

A total of 7 indigenous biocontrol agents were isolated from fructoplane of healthy pomegranate fruits. Initial antagonistic mycoflora were isolated on *Trichoderma* selective medium (TSM). Pure cultures of 7 *Trichoderma* spp. were obtained.

Pure cultures of *Trichoderma* sps. Isolated from fructoplane

Evaluation of native potential bio-agents:

In vitro evaluation was carried out with seven native potential bio-agents against DMC4 (Damalacheruvu) isolate of *Colletotrichum gloeosporioides* through dual culture technique.

Dual culture technique:

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus were inoculated at one end of the petriplate and antagonistic fungus was placed opposite to it on the other end. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The percentage inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent [6].

I= (C-T)/CX100

Where,

I = per cent inhibition
C = growth in control
T = growth in treatment

Results and discussion:

The pathogenicity test of *Colletotrichum gloeosporioides* to pomegranate was proved by Fruit inoculation method carried out under *in vitro* conditions. Control was maintained with sterilized healthy fruits. During advanced stage of infection the white mycelium grew around the inoculated region and completely covered it. The fungus was re-isolated from affected fruit part and compared with the original culture.

The seven antagonistic fungal isolates were evaluated by dual culture technique for their antagonistic effect against *Colletotrichum gloeosporioides* under *in-vitro* conditions. Inhibition zone in mm was recorded and the per cent inhibition was calculated. At 7 days after inoculation maximum inhibition of mycelial growth 73.50 per cent was observed in *T. harzianum* (Madanapale field isolate MDPF-1), which was followed by *T. viride* (Palamner field isolate PLRF-5) 60.43 per cent and least inhibition was recorded in *T. harzianum* (Punganur field isolate PGRF-3)29.70 percent respectively.

List of bio-agents used for *in vitro* evaluation against *Colletotrichum gloeosporioides* causing Anthracnose of Pomegranate by dual culture technique

S. No.	Bio control agents	Per cent inhibition of mycelial growth
1.	*Trichoderma harzianum* (DMCF-4)	58.00
2.	*Trichoderma viride* (RSDF-7)	53.33
3 .	***Trichoderma harzianum* (PGRF-3)**	**29.70**
4.	***Trichoderma viride* (PLRF-5)**	**60.43**
5.	*Trichoderma asperullum* (PTRF-2)	56.00
6.	***Trichoderma harzianum* (MDPF-1)**	**73.50**
7.	*Trichoderma harzianum* (KPMF-6)	54.30
8.	**Control**	**0.00**

In the present study the slow growth rate of the pathogen suggested a more rapid utilization of nutrients by the antagonists when grown together. Nutrient depletion, space and production of toxic substances (antibiotic and antibiotic like substances) by the fungi are known to play a dominant role in antagonism and these factors are usually governed by the physico- chemical nature of the environment [7]. The present in vitro study results showing the positive antagonistic effect of the fructoplane fungi which have restricted the growth of the pathogen under in vitro condition (i.e., *Trichoderma* sp.).Control of plant diseases by the use of antagonistic microorganisms can be an effective means [8]. Various plant diseases have been successfully controlled through bacterial and fungal antagonists [9]. (Cook and Baker, 1983; Campbell, 1989). Antagonistic fungal isolate such as *Trichoderma*, reduce growth, survival or infections caused by the pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion [10]. Biological control is an effective, ecofriendly and alternative approach for management of the disease.

Conclusions:

From the in vitro findings, it can be recommended that the antagonists *Trichoderma* species can be used as a bio-control agent against *Colletotrichum gloeosporioides* under field conditions. It is also exposed that these fungal isolates that naturally present on the fruit surfaces are having more or less similar potential antagonistic effect on the various crop disease caused by various pathogens. And some of them can be used as a potential bio- control agent under field condition to decrease the disease incidence and to increase crop yeild.

References:

1. Dennis C, Webster J. Antagonistic properties of groups of *Trichoderma* I. Production of non-volatile antibiotics. Trans. Brit. Mycol. Soc. 1971; 57:25-39.

2. Dutta BK. Studies on some fungi isolated from the rhizosphere to tomato plants and the consequent prospect for the control of *Verticillium* wilt. Pl. Soil, 1981; 63:209-216.

3. Upadhay JP, Mukhyopadhayay AN. Biological control of *Sclerotium rolfsii* by *Trichoderma harzianum* in Sugrbeet. Indian Phytopathol. 1986; 39:394-396.

4. Campbell R. Biological control of microbial plant pathogen. Cambridge University Press, Cambridge, 1989, 8-24. 8.

5. Rangaswami G. Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, 1988, 498.

6. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947; 150:850.

7. Burgess LW, Griffin DM. Competitive saprophytic colonization of wheat straw. Annu. Appl. Biol. 1967; 60:137-42.

8. Ekefan EJA, Gowen SR. Potential of *Trichoderma harzianium* isolates in biocontrol of *Colletotrichum capsici* causing anthracnoac of Pepper (*Capsicum* spp) in Nigeria. J Appl. Biosci. 2009; 20:1138-1145.

9. Cook RJ. Making greater use of introduced microorganisms for biological control of plant pathogens. Annu. Rev. Phytopathol. 1993; 31:53-80.

10. Cook RJ, Baker KF. The nature and practice of biological control of plant pathogens. In: American Phytopathological Society St. Paul, Minnesota, 1983, 539.