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BIOLOGICAL CONTROL OF FRUIT ROTS OF GRAPES CAUSED BY AUREOFUNGIN RESISTANT *ALTERNARIA TENIUS*

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Abstract- All the 16 fungal species from the phyllosphere, flower and the grape berries were tested for their antagonism against the resistant mutant of *Alternaria tenius* (At-EMS-3) *Glaocladium roseum* and two bacterial isolates gave antagonistic reactions. Application of cultural filtrates from one of the bacterium was more useful for the management of pathogen on the berries when compared with dual culture or only the bacterial cells.
Key words- Biological control, Antagonism, At-EMS-3

Introduction- Grape (*Vitis venifera* L.) is one of the very important fruit crop in India and Abroad. Grape plant become victim to many diseases in the life span but fruit rot is most important disease in the orchard and also in storage or transport. (Chahal and Mahli, 1969). Fruit rot of grapes caused by many fungi of these *Alternaria tenius* fruit rot is most important one (Krishnaiah et al., 1983; Rao,1993,1994) fruit rot decreases market value of the grape fruit hence causing large economic loss to the farmer. The control of fruit rot of grapes has been almost exclusively based opn the application of chemical fungicides, but use of fungicides is considered to be long term solution due to concern of expense risks, fungicide residue and other health and environmental hazards. Use of biocontrol agents has been shown to be ecofriendly and effective against many plant pathogens. It has been reported that *Fusarium sambucilum* (Kilkov et al., 1983), *Trichothecium roseum* (Dragoescu, 1986) prevent leaf and stem blight caused by *Alternaria solani*. The *Streptomyces* species and *Bacillius sultilis* (Mateascu et al., 2002; Sharma and Sharma, 2006; Sid et al., 2005) were also used to inhibit mycelial growth and spore germination of *Alternaria solani*. Hence attempt was made to find out the biological control of *Alternaria tenius* causing fruit rot of grapes.

Materials And Methods- Isolates of different microbes were collected from fruits flower and phyllosphere of grape plant (*Vitis venifera* L.) on Czapek-Dox agar medium poured in the petri plates. The resistant mutant of *Alternaria tenius* (*At-EMS-3*) was inoculated on culture plates. Isolates of different fungi and bacteria were inoculated near *Alternaria tenius* on same petri-plate and antagonistic reaction was observed.

Bacteria (*Bacillus* sp) were highly antagonistic to *Alternaria tenius* so bacteria were used as Biological control for further studies.

In vitro effect of bacterial cultural filtrate on resistant mutant of *Alternaria tenius* was observed by zone of inhibition. Antagonistic bacteria (*Bacillus sp*) were grown inside the nutrient broth medium in 100ml conical flask. Seven days after inoculation the cultural filtrate was obtained through cintered glass filter. Czapek-Dox agar medium poured in the sterilized petri plates and after solidification spore suspension of resistant mutant of *Alternaria tenius* was spread on the surface of agar medium with the help of L shaped rod. A well was in the centre of agar medium with the help of cock borer (8mm diameter) 0.1 ml culture filtrate was poured in the well. After seven days of incubation zone of inhibition were observed and these zones were measured.

In vivo – Bacterial culture filtrates were obtained as above. Grape berries were washed 10 times with sterile distilled water. The berries treated with bacterial culture filtrate and inoculated with spore suspension of resistant mutant of *Alternaria tenius.* These inoculated grapes were incubated at 26±3^{o}C in moist Petri-plates and PDI was calculated. Without bacterial culture filtrate and bacterial cells were also used for treatment of grape berries and PDI was calculated.

Result And Discussion- A total of 16 fungal species isolated from various sources such as phyllospere, flower and grape barriers were tested against *Alternaria tenius* resistant mutant At- EMS – 3 on agar by spot inoculums plates. Various associative effects were seen. Results in Table-1 show that *Penicillium funiculosum, Aspergillus fumigates, Curvularia* sp*., Helminthosporeum* sp*.and Nigrospora* sp. did not show any harmful effect

against each other. *Alternaria tenius* mutant overlapped on *Penicillium varians, Aspergillus flavus, Aspergillus niger Chaetomium sp. and Cunnighamella sp.* On the other hand *Rhizopus sp., Cladosporium* sp., *Trichoderma* sp. Overlapped on *Alternaria tenius* mutant. *Gliocladium roseum* and two bacterial isolates indicated antagonistic zone towards *Alternaria tenius* (Plate I). One of the bacterial isolates was used for biological control of *A. tenius* mutants on agar plate and also on grape berries.

Effects of bacterial culture filtrate on resistant mutant of *A. tenius* was observed by zone inhibition. Result in Table-2 and Plate II showed that inhibitory zone of bacterial culture filtrate was useful for the management of pathogen. The inhibitory zone of the two bacterial culture filtrates used were ranged from 11.5 to 13.5. Results in Table-3 showed that application of culture filtrate was more useful for management of this pathogen on the berries when compared with the dual culture and/or with the bacterial cells.

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***Table 1**: Antagonistic and associate relationship between Alternaria tenius and other phyllosphere, flower and berries fungi of grapes.*

Sr no.	Name of the fungi	Reaction A	Reaction B	Reaction C	Reaction D
1.	*Penicillium funiculosum*	+	-	-	-
2.	*Penicillium varians*	-	+	-	-
3.	*Aspergillus flavus*	-	+	-	-
4.	*Aspergillus niger*	-	+	-	-
5.	*Aspergillus fumigates*	+	-	-	-
6.	*Curvularia sp.*	+	-	-	-
7.	*Heminthosporium sp.*	+	-	-	-
8.	*Nigrospora sp.*	+	-	-	-
9.	*Chaetomium sp.*	-	+	-	-
10.	*Cunninghamella sp.*	-	+	-	-
11.	*Cladosporium sp.*	-	-	+	-
12.	*Rhizopus sp.*	-	-	+	-
13.	*Trichoderma sp.*	-	-	+	-
14.	*Gliocladium roseum sp.*	-	-	-	+ (2)
15.	*Bacillus sp.*	-	-	-	+ (3)
16.	*Bacillus Sp.*	-	-	-	+ (3)

A = Both colonies grow side by side without harming each other
B = Alternaria tenius overlaps on other fungi.
C = Other fungi overlaps on Alternaria tenius.
D = Other fungi antagonistic to Alternaria tenius.
**Figures in parentyhesis show antagonistic zone in mm.*

Table 2*-Inhibitory action of bacterial culture filtrate against Alternaria tenius (At- EMS-3) by cup plate method.*

Sr.No.	Bacterial culture filtrate	Inhibitory zone(mm)
1.	*Bacillus* spp.	11.5
2.	*Bacillus* spp.	13.5

Table 3*- Percentage control efficacy (PCE), Bacillus sp. Culture filtrate against Aureofungin resistant mutant of Alternaria tenius on grape berries.*

Bacterial sources	PCE
Culture filtrate	84.21
Dual culture	71.10
Bacterial cells	59.89
S.E.	5.73
C.D. at 0.05	81.14
C.D. at 0.01	85.04

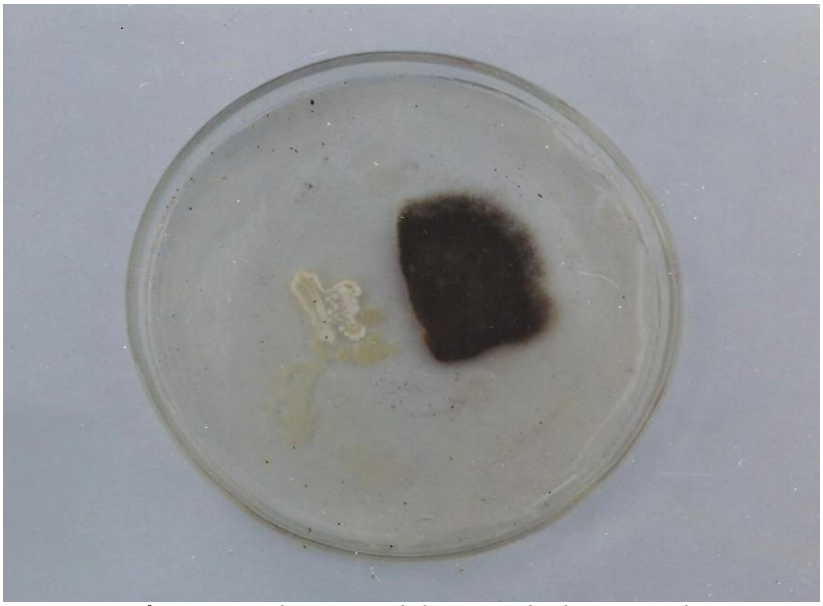

Plate- I- Bacterium antagonistic to *A. tenius* (At- EMS – 3)

Plate- II- Inhibition zone of Bacterial culture filtrates to antagonistic to *A. tenius* (At- EMS – 3) on Agar plates