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STUDIES ON SENSITIVITY OF *ALTERNARIA ALTERNATA* ISOLATES AGAINST AUREOFUNGIN

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Abstract- Sensitivity of 20 *Alternaria alternata* isolates causing fruit of grapes against Aureofungin was tested *in vitro* and *in vivo*. There was considerable variation in the sensitivity of the isolates. Aa – 1 was highly sensitive while isolate Aa – 19 was resistant to the application of Aureofungin. This was proved both on the agar plates and on the grape berries as well.
Keywords- *Alternaria alternata*, Aureofungin, fruit rot, Sensitivity.

Introduction

Fruit rot of grapes (*Vitis venifera* L.) caused by *Alternaria alternata is* an important disease in Maharashtra (Chahal and Malhi 1969, Rao 1994). This pathogen infects the fruit near the pedicle and spread all over the fruit. The spores are carried in the storage and transport spoils the market value of fruits. Various antibiotics and systematic fungicides are used to control these diseases. But development of resistance against these antibiotics and systematic fungicides was recorded (Ito and Yamegushi, 1977; Dekker, 1982; Gangawane, 1990) Barkai et al. (2002) checked sensitivity of melon fruit storage fungi to the gamma irradiations and concluded that *Alternaria tenius* is radiation resistant both *in vitro* and *in vivo*. Rosenweig et al. (2008) has checked sensitivity of *Alternaria solani* to the different fungicides. Blixt and Anderson (2010) have studied sensitivity of *Alternaria solani* to the Strobilurins. Aureofungin is one of the antibiotics recommended for the management of fruit rot disease of grapes. It was therefore decided to examine the possibility of development of Aureofungin resistance in the pathogen.

Materials and Methods

Twenty isolates of *Alternaria alternata* in few fruit of grapes were collected from the different orchards and markets of Maharashtra region and mounted on Czapek Dox agar medium and sensitivity of these isolates against Aureofungin was tasted *in vitro* and *in vivo*.

In vitro- Sensitivity test for Alternaria alternata twenty isolates isolated were carried out against Aureofungin with the help of food poisoning technique. This was done by studying radial growth of *Alternaria alternata* isolates on agar plates containing fungicides (Dekker and Geeling, 1979). Czapak Dox agar medium (2x) was prepared. It was sterilized and 10 ml of this medium (Concentrated) selected for study in sterile properties. A series of concentration (100 to 2000gm/lit.) was prepared. The fungicide solutions were thoroughly mixed with medium and allow solidifying. A 4 mm disc of the isolates from 7 days old culture plates were transferred aseptically at the center of Petri-plates. The plates in the triplicates were at 26 ± 3^{0}C in the dark and linear growth was measured every day up to 8 days MIC and Fungicide concentration with 50 % reduction in radial growth (ED_{50}) of 20 isolates was thus determined. This data was subjected to statistical analysis on the computer. MIC and ED_{50} values were calculated by using formula given by Molnar et. al. 1985

In vivo: This was done by studying PDI on grape barriers. Grape barriers washed 10 times with sterile distilled water then this grape barriers treated with Aureofungin at different concentration (100 µg/ml to 2000 µg/ml). Then this barriers isolated with spores suspension of *Alternaria alternata* by pin prick method and kept in moist Petri-plates for inoculation at 26 ± 3^{0}C in the dark and PDI was calculated after 8 days using following formula-

$$PDI = \frac{\text{Of All Rating}}{\text{Total No. of barriers observed x 4}} \text{ X } 100$$

Result

In vitro: Sensitivity of *Alternaria alternata in vitro* was checked and results were tabulated in Table-1. It was noted that isolate Aa-1 was highly sensitive MIC (344.89 µg/ml). Isolate Aa-2, Aa-3, Aa-6, Aa-7, Aa-10 and Aa-12 were inhibited at 362 to 500 µg/ml other 12 were

inhibited at the concentration of 500 to 835.88 µg/ml). It was recorded that only one isolate (Aa-19) was resistant. *In vivo*: Sensitivity of *Alternaria alternata in vivo* was checked and results were tabulated in Table-2. It indicates that MIC ranged from 527.65 µg/ml to 1528.18 µg/ml. Agar isolate Aa-1 was more sensitive to Aureofungin. Isolate Aa- 19 was more resistant to the Aureofungin. Isolate Aa-15, Aa-17, and Aa-18 were inhibited at more than 1000 µg/ml concentration of Aureofungin. MIC for other isolates ranged in between 578.72 µg/ml - 965.75 µg/ml

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Table1: Sensitivity of Alternaria alternata isolates against Aureofungin on agar plates

Isolates	Reg. Const.	Reg. Coeff.	Corr. Coeff.	ED_{50}	MIC
Aa-1	-3.8734	0.0083	0.9985	143.13	324.89
Aa-2	-3.5422	0.0112	0.9854	165.24	362.27
Aa-3	-3.5832	0.0099	0.9919	215.20	486.32
Aa-4	-3.6052	0.0086	0.9848	236.31	537.65
Aa-5	-3.5234	0.0109	0.9796	223.13	537.07
Aa-6	-2.8556	0.0141	0.9660	258.54	465.95
Aa-7	-3.4367	0.0092	0.9758	156.61	347.86
Aa-8	-3.1372	0.0089	0.9812	294.82	627.54
Aa-9	-2.8851	0.0105	0.9663	261.81	603.52
Aa-10	-2.7207	0.0155	0.9613	364.21	756.25
Aa-11	-3.1640	0.0093	0.9843	196.34	436.16
Aa-12	-3.3856	0.0074	0.9809	182.11	388.58
Aa-13	-3.3204	0.0089	0.9741	326.64	678.94
Aa-14	-2.5948	0.0112	0.9775	231.72	578.66
Aa-15	-3.5874	0.0100	0.9925	323.18	791.51
Aa-16	-4.2273	0.0079	0.9937	308.86	714.66
Aa-17	-3.5369	0.0112	0.9857	418.08	853.88
Aa-18	-3.5510	0.0108	0.9738	363.27	784.25
Aa-19	-2.9402	0.0115	0.9645	437.35	974.74
Aa-20	-3.6052	0.0086	0.9848	298.22	648.73

ED_{50} - Fungicide concentration with 50% reduction in radial growth
MIC - Minimal inhibitory concentration

Table 2: Sensitivity of Alternaria alternata isolates against Aureofungin on grape berries

Isolates	Reg. Const.	Reg. Coeff.	Corr. Coeff.	ED_{50}	MIC
Aa-1	-2.9402	0.0115	0.9645	246.38	527.65
Aa-2	-2.6674	0.0205	0.9487	264.24	578.72
Aa-3	-2.8556	0.0141	0.9660	303.48	717.94
Aa-4	-2.9644	0.0139	0.9596	338.93	768.91
Aa-5	-2.8143	0.0207	0.9451	307.99	714.66
Aa-6	-2.9580	0.0110	0.9508	396.78	803.06
Aa-7	-3.2013	0.0090	0.9410	241.72	558.23
Aa-8	-26674	0.0205	0.9487	386.23	869.67
Aa-9	-1.6325	0.0034	0.8932	336.26	884.63
Aa-10	-2.0740	0.0250	0.8677	381.37	944.63
Aa-11	-2.9951	0.0139	0.9535	313.85	678.27
Aa-12	-1.1683	0.0081	0.8775	294.67	603.58
Aa-13	-1.3303	0.0113	0.8484	436.34	965.74
Aa-14	-1.2467	0.0103	0.8643	326.21	761.51
Aa-15	-2.6092	0.0212	0.9551	496.32	1049.23
Aa-16	-1.2176	0.0076	0.8425	396.54	926.45
Aa-17	-1.2473	0.0114	0.8346	549.95	1197.75
Aa-18	-2.9513	0.0146	0.9628	507.37	1087.93
Aa-19	-2.9402	0.0115	0.9645	649.36	1528.18
Aa-20	-3.2513	0.0220	0.9531	378.34	914.74

ED_{50} - Fungicide concentration with 50% reduction in radial growth
MIC - Minimal inhibitory concentration

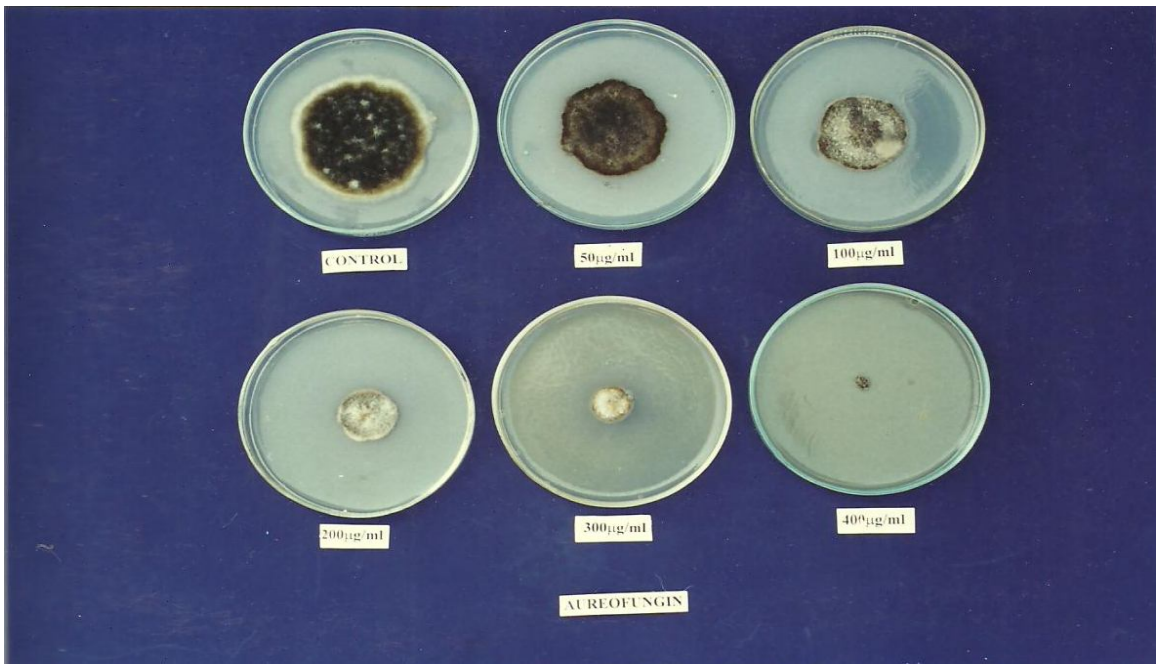

Plate- I- Sensitivity if *Alternaria alternata* isolates against aureofungin on Czapek – Dox agar plates.