



Synergistic effect of Antibiotics on Aureofungin resistance in *Alternaria tenuis* causing fruit rot of grape

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Abstract

Fruit rot of grape caused by *Alternaria tenuis* was found to be resistant to Aureofungin. Antibiotics viz. Griseofulvin, Mycostatin, Penicillin and Streptomycin individually or in mixture with Aureofungin were tested both *in vitro* and *in vivo* against resistant mutant of *Alternaria tenuis*. Results showed that individually PCE was higher in Mycostatin followed by Griseofulvin, streptomycin and Penicillin. Use of Aureofungin mixed with antibiotics, the PCE was highly increased.

INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the very important fruit crop in India and abroad. However, fruit rot of Grapes is caused by many fungal pathogens. Among these, Fruit rot of grape caused by *Alternaria tenuis* is destructive disease in the field as well as during storage and transport (Chahal and Malhi, 1969; Krishnaiah *et al.*, 1983; Rao, 1994). Aureofungin is most effective fungicide against *Alternaria spp.* (Ghosh and Gemawat, 1976; Krishna *et al.*, 1998). Fungicide resistant cases in various plant pathogens have been reported in India as well as in other countries (Wild, 1980; Annamalai and Lalithakumari, 1990; Gangawane *et al.*, 1995). The main objective of present study was to find out the synergistic effect of antibiotics on the management of Aureofungin resistant mutant of *Alternaria tenuis*.

MATERIALS AND METHODS

In vitro study was undertaken on Czapek Dox agar medium while *in vivo* on grape fruits. For this purpose twenty isolates of *Alternaria tenuis* were collected from fields and markets of different regions of Maharashtra. The sensitivity of these

isolates of *Alternaria tenuis* to aureofungin was tested by using food poisoning technique (Nene & Thaplial, 1993). Czapek Dox agar plates containing different concentration (50 – 1000 µg/ml) of Aureofungin were prepared. Disc (4mm) of pathogen isolates taken from the margin of 7 days old colony were placed in the center of agar plates. These plates were then incubated at 26±3°C and linear growth was measured at different intervals up to a week. MIC and ED₅₀ were calculated. Thus the sensitivity of twenty isolates was determined. There was a large variation in the sensitivity of isolates. During present investigation, disease resistance of the pathogen was developed by chemical mutation and it was used for further study as suggested by Dekker (1982). Thus the EMS-At-3 mutant was obtained with highest resistant factor 6 and used for present study. The agar plates containing antibiotic individually and in mixture of Aureofungin and antibiotics (10, 50 and 100 µg/ml) were prepared and inoculated with resistant mutant. The plates were incubated at 26±1°C. The agar plates without treatment served as control. The percentage control efficacy (PCE) was calculated 8 days after incubation period by using following equation

$$PCE = 100 (1 - x/y)$$

Where, x = Diameter of colony in treated plates or Percentage disease index of treated fruits

y = Diameter of colony in control or Percentage disease index of untreated fruits.

In vivo studies were carried out on fruit of grape. The fruits were surface sterilized by treating them with 1% HgCl₂ solution and were washed ten times with sterilized distilled water. The fruits were then treated with antibiotics individually and in mixture of Aureofungin and antibiotics (50 and 100 µg/ml). The resistant mutant *Alternaria tenuis* was

inoculated by pin prick method on the fruits and they were incubated for a week at 26 ± 3°C in the laboratory. Fruits without treatment served as control. Percentage disease index (PDI) was calculated as described by Datar and Mayee (1985) and then on PDI, the percentage control efficacy was calculated as above equation.

Table 1. Percentage control efficacy (PCE) of Antibiotics individually and in mixture with aureofungin against aureofungin resistant mutant (EMS-At-3) of *Alternaria tenuis* on agar plates.

| Sr. No. | Antibiotics (µg/ml) | Individual (PCE) | Mixture (PCE) |
|---------|-------------------------|------------------|---------------|
| 1 | Griseofulvin 10 | 32.20 | 51.16 |
| | 50 | 46.78 | 67.32 |
| | 100 | 64.74 | 76.04 |
| 2 | Mycostatin 10 | 37.88 | 56.76 |
| | 50 | 54.71 | 70.12 |
| | 100 | 68.44 | 83.42 |
| 3 | Penicillium 10 | 13.25 | 46.29 |
| | 50 | 28.51 | 58.33 |
| | 100 | 43.14 | 64.47 |
| 4 | Streptomycin 10 | 22.43 | 52.34 |
| | 50 | 34.13 | 62.15 |
| | 100 | 58.66 | 69.37 |
| 6 | Aureofungin (800 µg/ml) | 44.67 | - |
| | S. E. | 4.38 | 2.99 |
| | C.D. at 0.05 | 49.46 | 68.53 |
| | 0.01 | 52.43 | 70.75 |

Table 2. Percentage control efficacy (PCE) of Antibiotics individually and in mixture with aureofungin against aureofungin resistant mutant (EMS-At-3) of *Alternaria tenuis* on grape fruits.

| Sr. No. | Antibiotics (µg/ml) | Individual (PCE) | Mixture (PCE) |
|---------|-------------------------|------------------|---------------|
| 1 | Griseofulvin 50 | 34.26 | 53.26 |
| | 100 | 46.24 | 65.49 |
| 2 | Mycostatin 50 | 48.79 | 57.65 |
| | 100 | 56.25 | 70.00 |
| 3 | Penicillium 50 | 18.26 | 44.25 |
| | 100 | 31.43 | 52.60 |
| 4 | Streptomycin 50 | 21.15 | 47.33 |
| | 100 | 38.24 | 58.64 |
| 6 | Aureofungin (800 µg/ml) | 38.04 | - |
| | S. E. | 3.91 | 2.85 |
| | C.D. at 0.05 | 43.48 | 60.85 |
| | 0.01 | 46.13 | 66.64 |

RESULTS AND DISCUSSION

In vitro and *in vivo* present study revealed that when antibiotics tested individually against *Alternaria tenuis* causes fruit rot of grapes, Mycostatin was found to be higher PCE followed by Griseofulvin, streptomycin and Penicillin. But when Aureofungin was used in mixture with antibiotics, the PCE was highly increased (Table 1 and Table 2). Use of aureofungin in mixture with antibiotics was more effective.

The results are in agreement with the finding of earlier workers, Khilare et al (2001) reported that use of thiophanate methyl in combination with antibiotics against *Penicillium digitatum*, mycostatin gave higher PCE. Gangawane L.V. and Kamble S.S. (2001) found that when carbendazim was used in combination with agrochemicals inhibited the growth of resistant isolate of *Macrophomina phaseolina* causing charcoal rot of potato. Bhale et al. (2009) reported that when use of fungicide in mixture with antibiotics against *Fusarium oxysporum*, ampicillin is more effective. Dekker, 1981; Gangawane and Readdy, 1987 suggested that there is significant delay of resistance build up in the pathogen when the mixture of different agrochemicals was used.

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