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### RESEARCH ARTICLE

## SENSITIVITY OF ALTERNARIA ALTERNATA AGAINST CARBENDAZIM CAUSING FRUIT ROT OF POMEGRANATE.

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#### Abstract

It was found that there was variation in the MIC of carbendazim among 21 isolates of *Alternaria alternata* (Fr.) Keissler on agar plates and on pomegranate fruits. MIC on agar plates ranged from 500 to 1000 µg/ml and Ed<sub>50</sub> from 325.33 to 693.75 µg/ml, while it was 100 to 350 µg/ml and Ed<sub>50</sub> 33.76 to 136.48 µg/ml, on fruits Collected from Kolar (Dist. Ahmednagar), Parali (Dist. Beed), Mahabaleshwar (Dist. Satara), Rahuri (Dist. Ahmednagar) and Sangli. It appears that with the increase of the concentration of fungicide in the medium and on the pomegranate fruits there was decrease in the radial growth of the pathogen.

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#### Introduction:-

Pomegranate (*Punica granatum* L.) is one of the important fruits in our country. It is grown in tropical and sub-tropical regions of the world. Pomegranate is grown as commercial crop in India. In Maharashtra many farmers are growing pomegranate. Pomegranate fruits are taken in all the three seasons but recently due to change in climatic conditions and storage the crop is attacked by many fungal pathogens (Padule and Kaulgud, 1993). Among the fungal pathogens *Alternaria alternata* was very common (Bharade and Kamble, 2001). The disease caused by *Alternaria alternata* to pomegranate fruit is managed by using carbendazim. The present investigation was carried out to determine the minimum inhibitory concentration of carbendazim for effective disease management.

#### Material and Method:-

Twenty one Fruit samples exhibiting fruit rot of pomegranate were collected from different fruit markets and surrounding of pomegranate fields of Ahmednagar, Rahuri, Kolhapur, Aurangabad, Sangli, Parbhani, Gangakhed, Kolhapur, Beed, Jalna, Pandharpur, Shrirampur, Yavatmal, Latur (Ausa), Parali, Mahabaleshwar and Sangli districts of Maharashtra. To isolate the causal agent, they were brought to the laboratory in clean sterilized polythene bags. The infected portion of fruit is cut in to the size 2 mm and sterilized by using 0.1% HgCl<sub>2</sub> and washed with sterilized distilled water (Jadhav et al., 2010), these sterilized fruit portion were kept on a Czapek dox agar plates amended with streptomycin sulphate (Patil et al., 2012; Mali et al., 2015). Inoculated plates were incubated at 28 ± 20 C for growth of the fungus and further studies (Mali et al., 2016). After 9-10 days of culture, greyish fungal mass was observed. On the basis of morphological, microscopic characters and following relevant mycological literature the fungal isolate was identified as *Alternaria alternata* (Fr.) Keissler. In this manner, 21 isolates were obtained. In vitro and in vivo sensitivity (MIC) of *Alternaria alternata* (Fr.) Keissler and ED<sub>50</sub> of carbendazim was carried out by

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using Food Poisoning Technique (Dekker and Gielink, 1979). Czapek Dox agar medium plates were prepared containing different concentrations of carbendazim. After solidification of media, a disc (8 mm) with fungal culture was obtained from the margin of an actively growing colony and placed upside down on the agar surface. These plates were then incubated at 28-30°C in 12 hour cycle of dark and light and then continuous growth was measured after various time intervals. Plates without carbendazim was served as control. On pomegranate fruits 8 mm diameter hole having 3 cm depth was prepared with the help of cork borer and was inoculated with spore suspension of various isolates along with different concentrations (a.i) of carbendazim solution treated before 24 hours. Pomegranate fruits were wrapped with moist paper towels and percent infection was recorded after various incubation periods. From Table 1. and Table 2. again proved that there are six groups in the isolates according to their sensitivity viz., Group – I (Aa–2), Group – II (Aa–3, Aa–4) Group III (Aa–1, Aa–5, Aa–9, Aa–10, Aa–11, Aa–14), Group IV (Aa–6, Aa–7, Aa–8, Aa–12, Aa–13), Group V (Aa–15), Group VI (Aa–16, Aa–17, Aa–18, Aa–19, Aa–20, Aa–21). Group first indicates MIC 500 µg/ml, Group – II 600 µg/ml, Group – III 700 µg/ml, Group – IV 800 µg/ml, Group V 900 µg/ml and Group – VI 1000 µg/ml. Similarly in vivo Group I showed MIC 100 µg/ml, Group–II 140 – 150 µg/ml, Group–III 200 and group – IV 350 µg/ml MIC respectively. The dose response curves were calculated by means of computer using equation.

$$Y = \frac{H}{1 + \text{Exp} (at bx)}$$

Where,

1. Y = radial growth as percent of control
2. H = upper limit of the curve.
3. Exp. = logarithmic exponent
4. a = regression constant
5. b = regression coefficient
6. x = measured parts by (Molnar et al., 1985).

### Result and Discussion:-

Results are given in Tables 2 and Table 3. It was seen that there was variation in MIC of carbendazim against fruit rot pathogen on agar plates and on pomegranate fruits. MIC on agar plates ranged from 500 to 1000 µg/ml and Ed<sub>50</sub> from 325.33 to 693.75 µg/ml, while it was 100 to 350 µg/ml and Ed<sub>50</sub> 33.76 to 136.48 µg/ml. Fig 1 and Fig 2 reveals that with the increase of the concentration of fungicide in the medium and on the pomegranate fruits there was decrease in the radial growth of the pathogen. The same reports were observed by many workers (Van Tuyl, 1975), In India attention towards fungicide resistance is focused by Gangawane (1981), Annamalai and Lalithakumari (1990). Gangawane and Kamble (1993), Chander and Thind (1995). Putto and Chaudhary (1986).

**Table 2:-** MIC of Carbendazim against *Alternaria alternata* causing fruit rot of pomegranate in vitro

Sr. No.	Isolate	Data characteristic to dose response curve					
		Locality	Regression		Correlation coefficient	ED <sub>50</sub> * µg/ml	MIC** µg/ml
			Constant	Coefficient			
1	Aa-1	Aurangabad	-3.2202	0.0019	0.7735	352.33	700.00
2	Aa-2	Solapur	-3.7203	0.0113	0.8073	329.72	500.00
3	Aa-3	Sangavi	-4.3104	0.0127	0.8793	340.32	600.00
4	Aa-4	Parbhani	-3.7989	0.0117	0.8223	325.93	600.00
5	Aa-5	Chavani, A'bad	-3.1553	0.0092	0.7696	343.22	700.00
6	Aa-6	Osmanabad	-2.8832	0.0072	0.7391	397.79	800.00
7	Aa-7	Gangakhed	-2.7813	0.0072	0.7289	385.01	800.00
8	Aa-8	Kolhapur	-3.3629	0.0075	0.7880	451.00	800.00
9	Aa-9	Ahmednagar	-3.1926	0.0079	0.7887	406.34	800.00
10	Aa-10	Beed	-3.0543	0.0087	0.7443	349.50	700.00
11	Aa-11	Jalna	-3.1995	0.0085	0.7494	376.24	700.00
12	Aa-12	Pandharpur	-2.7520	0.0073	0.7283	377.46	800.00

13	Aa-13	Shrirampur	-3.0820	0.0075	0.7647	412.31	800.00
14	Aa-14	Yavatmal	-3.1706	0.0088	0.7562	361.83	700.00
15	Aa-15	Padegaon, A'bad	-2.7920	0.0060	0.7292	465.38	900.00
16	Aa-16	Ausa, Latur	-2.9349	0.0061	0.7494	477.41	900.00
17	Aa-17	Kolar	-2.8890	0.0059	0.7834	485.82	1000.00
18	Aa-18	Parali	-2.5417	0.0054	0.7269	473.64	1000.00
19	Aa-19	Mahabaleshwar	-2.9317	0.0055	0.7643	536.95	1000.00
20	Aa-20	Rahuri	-2.8951	0.0055	0.7669	521.74	1000.00
21	Aa-21	Sangali	-3.6508	0.0053	0.8089	693.75	1000.00

ED<sub>50</sub> Carbendazim cocentration causing 50% reduction in radial growth

MIC Minimum inhibitory concentration

**Table 3:-** MIC of Carbendazim against *Alternaria alternata* causing fruit rot of pomegranate in vivo

Sr. No.	Isolate	Data characteristic to dose response curve					
		Locality	Regression		Correlation coefficient	ED <sub>50</sub> * µg/ml	MIC** µg/ml
			Constant	Coefficient			
1	Aa-1	Aurangabad	-1.6655	0.0440	0.6325	37.87	100.00
2	Aa-2	Solapur	-1.3651	0.0404	0.5773	33.76	100.00
3	Aa-3	Sangavi	-2.1738	0.0516	0.7209	42.13	100.00
4	Aa-4	Parbhani	-1.6794	0.0472	0.6581	35.61	100.00
5	Aa-5	Chavani, A'bad	-2.3340	0.0508	0.7308	45.93	100.00
6	Aa-6	Osmanabad	-1.5336	0.0325	0.3562	47.14	140.00
7	Aa-7	Gangakhed	-1.4088	0.0312	0.6689	45.16	150.00
8	Aa-8	Kolhapur	-1.3621	0.0299	0.6518	45.49	150.00
9	Aa-9	Ahmednagar	-1.6155	0.0319	0.6852	50.60	150.00
10	Aa-10	Beed	-2.4788	0.0281	0.7727	88.36	200.00
11	Aa-11	Jalna	-2.6764	0.0295	0.7986	90.73	200.00
12	Aa-12	Pandharpur	-1.8287	0.0244	0.6806	74.87	200.00
13	Aa-13	Shrirampur	-1.9365	0.0262	0.7115	73.82	200.00
14	Aa-14	Yavatmal	-2.7703	0.0411	0.7537	67.47	140.00
15	Aa-15	Padegaon, A'bad	-2.7644	0.0292	0.8013	94.73	200.00
16	Aa-16	Ausa, Latur	-1.0641	0.0106	0.5828	100.66	350.00
17	Aa-17	Kolar	-1.0680	0.0105	0.5812	101.79	350.00
18	Aa-18	Parali	-0.9549	0.0104	0.5683	92.23	350.00
19	Aa-19	Mahabaleshwar	-1.7271	0.0127	0.6615	136.48	350.00
20	Aa-20	Rahuri	-1.1493	0.0109	0.6006	105.53	350.00
21	Aa-21	Sangali	-1.1262	0.0127	0.6504	108.50	350.00

\* ED<sub>50</sub> Carbendazim cocentration causing 50% reduction in radial growth

\*\* MIC Minimum inhibitory concentration

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