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In vitro evaluation of fungicides against *Colletotrichum gloeosporioides* causing fruit rot of custard apple

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Abstract
The importance of fruits in human diet has been well recognized. Custard apple is one of major important fruit crop grown in Maharashtra. The indigenous fruits which are locally available in a particular season play a vital role in the nutrition of rural mass. Though, it was considered hardy and resistant to various pests and diseases, the losses caused by fruit rot of custard apple have been increased during recent years. Losses even up to 60-70% have been reported by previous workers. Fungicide use to control disease is a common practice.

The present investigation was carried out to evaluate bio efficacy of fungicides, botanicals and bio agents against *Colletotrichum gloeosporioides in vitro*. Four systemic and two non-systemic fungicides were tested at three different concentrations *in vitro* against pathogen. Among these fungicides Copper oxychloride at all concentrations, Captan at half and recommended concentration and Fenamidon at recommended concentration inhibited cent per cent mycelial growth of the pathogen.

Keywords: Fungicides, custard apple, fruit rot disease and concentrations

Introduction
Custard apple (*Annona squamosa* L.) is a native of tropical America and widely distributed throughout the tropical and subtropical regions. It is grown on marginal lands and hillocks with minimum inputs (Rajput, 1985) [5]. Recently area under cultivation of custard apple is increasing due to high economic returns and great export potential. Farmers prefer this crop because it is very hardy and can be successfully grown even on shallow to light soils with low water requirement. It is also considered as devoid of diseases and pest but in recent years crop has found susceptible to various pests and diseases. Among the various diseases, fungal diseases play an important role to severe loss of custard apple production. About 60 - 70 per cent losses have been reported due to the fruit rot disease (Gaikwad, 2002) [1]. The market for fruits and its export potential is totally dependent on quality and appearance of fruits. The fruit rot disease impairs fruit quality and makes them unsuitable for market. This leads to high economic losses. Thus the studies were carried out to evaluate fungicides in *in vitro* against *Colletotrichum gloeosporioides.*

Material and Methods
The fungi responsible for causing fruit rot disease in custard apple was isolated from diseased fruits procured from All India Coordinated Research Project on Arid Zone Fruits (Fig and Custard apple), Jadhavwadi, Dist.-Pune. The pure culture was obtained and the experiment was conducted in Completely Randomized Design with seven treatments and three replications in pathology laboratory at College of Agriculture, Pune.

The fungicides were evaluated by poison food technique. The required quantity of fungicides was mixed in molten sterilized PDA medium and then sterile Petri plates were filled with about 20 ml poisoned medium. Fungal colony of 0.4 mm diameter was placed in each plate at centre of plate under aseptic condition. The plates were incubated at 28 ^{0}C in BOD incubator. The control set was provided by using plates without fungicides. The observations of fungal colony growth inhibition were taken after 10 days of inoculation. Per cent inhibition of mycelial growth of the fungus was calculated by using the formula of Vincent (1947) [10].

$$I = \frac{(C - T)}{C} * 100$$

C = Growth (mm) in control after ten days
T = Growth (mm) in treatment after ten days

Where, I = Per cent growth inhibition

Trade name	Common name	Chemical name	Source
Contact fungicides were evaluated at (0.025%, 0.05% and 0.1%)			
Blitox-50	Copper oxychloride 50 WP	Copper oxychloride containing 50% metallic copper	Rallis India Ltd., Mumbai
Captaf	Captan 50 WP	Phthalimide class of fungicide	Rallis India Ltd., Mumbai
Systemic fungicides were evaluated at (0.05%, 0.1% and 0.2%)			
Amistar	Azoxystrobin 23 SC	Methyl (2E)-2-(2-(6-(-cyanophenoxy) pyrmidin-4-yloxylphenyl) -3-methoxyacrylate	Syngenta India Ltd.
Indofil Z-78	Zineb 75% WP	Manganese containing dithiocarbamate	Indofil Industries Ltd.
Antracol	Propineb 70% WP	Zinc containing dithiocarbamate	Bayer Crop Science, SAS, France
Sectin 60 WG	Fenamidone 10%+ Mancozeb 50% WG	Fenamidone and Mancozeb containing fungicide	Bayer Crop Science, SAS, France

Results and Discussion

Six different fungicides were tested at normal, ½ and ¼ concentration of recommended dose *in vitro* for knowing their effectiveness in inhibiting the mycelia growth of the pathogen. Out of six different fungicides four were systemic and two were non-systemic in nature.

In vitro bio-efficacy of fungicides at ¼ concentration against *C. gloeosporioides*

Significant results were obtained from these tests as presented in table 1. At lowest (1/4) concentration only Copper oxychloride inhibited cent per cent growth of pathogen. The next effective treatment was Captan in which 89.67 per cent growth inhibition was observed. While rest of the fungicides showed Zineb (9.22%), Propineb (20.66%), Fenamidon (57.93%) and Azoxystrobin (17.71%) growth inhibition of the pathogen.

In vitro bio-efficacy of fungicides at ½ concentration against *C. gloeosporioides*

At half dose of fungicides in captan and copper oxychloride no mycelial growth of the pathogen was observed where these fungicides inhibited cent per cent growth of the fungus (Table 2). Fenamidon was found next effective treatment with 92.99 per cent inhibition of pathogen. Rest of the fungicides showed inhibition of mycelia growth of pathogen as 23.25 per cent, 39.85 per cent and 22.87 per cent in Zineb, Propineb and Azoxystrobin fungicides, respectively

In vitro bio-efficacy of fungicides at recommended concentration against *C. gloeosporioides*

The data presented in table 3 revealed that there was cent per cent inhibition of mycelial growth of *C. gloeosporioides* at recommended concentration in three fungicides *viz;* fenamidon, captan and copper oxychloride while other fungicides *viz;* Zineb, Propineb and Azoxystrobin inhibited mycelial growth of pathogen (31.36%, 85.61% and 23.25%) respectively

The findings are closer to the work of Patil *et al.* (2009) [4] who reported the mycelia inhibition (64.88%) of *C. gloeosporioides* in treatment copper oxychloride (0.2%). The results are matching with the report of Tasiwal *et al.* (2008) [9] who noticed that non-systemic fungicide captan (0.15%) inhibited (84.09%) the mycelia growth of *C. gloeosporioides* of papaya anthracnose. The results of present findings are not matching with the finding of earlier workers like Singh *et al.* (2008) [6], Sivakumar *et al.* (2016) [7], Kumari Pavitra *et al.* (2017) [3] and Stanley *et al.* (2018) [8] where they found the copper oxychloride is least effective in inhibition of mycelia growth of the pathogen.

Table 1: *In vitro* bio-efficacy of fungicides at ¼ the concentration against *C. gloeosporioides*

Sr. No.	Fungicides	Conc. (%)	Colony diameter (cm) and growth rate i.e. GR (mm hr^{-1}) - hours after inoculation						Growth	
			48	96	144	192	240	Mean GR	Degree	Inhibition (%)
1	Zineb	0.25	1.33	3.13	5.40	6.73	8.20		++++	9.22
			0.28	0.38	0.47	0.28	0.30	0.34		
2	Propineb	0.25	1.27	3.07	4.37	5.33	7.17		+++	20.66
			0.26	0.38	0.27	0.20	0.38	0.30		
3	Fenamidon	0.25	0.47	1.27	2.47	2.90	3.80		++	57.93
			0.09	0.17	0.25	0.09	0.19	0.16		
4	Azoxystrobin	0.25	1.00	3.10	5.20	6.13	7.43		+++	17.71
			0.21	0.44	0.44	0.20	0.27	0.31		
5	Captan	0.5	0.43	0.53	0.63	0.93	0.93		+	89.67
			0.09	0.02	0.02	0.06	0.00	0.04		
6	COC	0.5	0.00	0.00	0.00	0.00	0.00		-	100.00
			0.00	0.00	0.00	0.00	0.00	0.00		
7	Control		1.50	3.80	6.67	8.67	9.03		++++	-
			0.31	0.48	0.59	0.42	0.07	0.38		
	S.E +		0.06	0.14	0.24	0.10	0.07			
			0.01	0.03	0.03	0.05	0.02			
	C.D. (0.05)		0.19	0.44	0.72	0.30	0.20			

			0.04	0.08	0.10	0.14	0.05			
	CV %		12.32	12.04	11.73	3.93	2.24			
			11.93	19.18	18.53	42.75	17.68			

Note: 1. Figures in bold faces indicate growth rate (mm hr^{-1}) values.
2. Degree of mycelial growth : - NIL, +Poor, ++ Moderate, +++ Good and ++++ Adundant

Table 2: *In vitro* bio-efficacy of fungicides at ½ concentration against *C. gloeosporioides*

Sr. No.	Fungicides	Conc. (%)	Colony diameter (cm) and growth rate i.e. GR (mm hr^{-1}) - hours after inoculation						Growth	
			48	96	144	192	240	Mean GR	Degree	Inhibition (%)
1	Zineb	0.5	1.03	2.27	4.10	5.10	6.93		++	23.25
			0.22	0.26	0.38	0.21	0.38	0.29		
2	Propineb	0.5	0.63	1.23	2.83	3.50	5.43		++	39.85
			0.13	0.12	0.34	0.14	0.40	0.23		
3	Fenamidon	0.5	0.40	0.43	0.43	0.50	0.63		+	92.99
			0.08	0.01	0.00	0.01	0.03	0.03		
4	Azoxystrobin	0.5	1.20	3.10	5.30	6.17	6.97		++	22.87
			0.25	0.40	0.46	0.18	0.17	0.29		
5	Captan	1	0.00	0.00	0.00	0.00	0.00		-	100.00
			0.00	0.00	0.00	0.00	0.00	0.00		
6	COC	1	0.00	0.00	0.00	0.00	0.00		-	100.00
			0.00	0.00	0.00	0.00	0.00	0.00		
7	Control		1.50	3.80	6.67	8.67	9.03		+++	0.00
			0.31	0.48	0.59	0.42	0.07	0.38		
	S.E +		0.11	0.20	0.29	0.33	0.32			
			0.02	0.02	0.05	0.03	0.04			
	C.D. (0.05)		0.33	0.62	0.87	1.00	0.97			
			0.07	0.07	0.15	0.08	0.11			
	CV %		27.00	22.52	17.84	16.57	13.18			
			26.41	21.61	32.41	34.45	41.12			

Note: 1. Figures in bold faces indicate growth rate (mm hr^{-1}) values.
2. Degree of mycelial growth : - NIL, +Poor, ++ Moderate, +++ Good and ++++ Adundant

Table 3: *In vitro* bio-efficacy of fungicides at recommended concentration against *C. gloeosporioides*

Sr. No.	Fungicides	Conc. (%)	Colony diameter (cm) and growth rate i.e. GR (mm hr^{-1}) - hours after inoculation						Growth	
			48	96	144	192	240	Mean GR	Degree	Inhibition (%)
1	Zineb	1	1.30	2.70	4.17	5.10	6.20		++	31.36
			0.27	0.29	0.30	0.19	0.23	0.26		
2	Propineb	1	0.40	0.53	0.67	0.83	1.30		+	85.61
			0.08	0.03	0.03	0.03	0.10	0.05		
3	Fenamidon	1	0.00	0.00	0.00	0.00	0.00		-	100.00
			0.00	0.00	0.00	0.00	0.00	0.00		
4	Azoxystrobin	1	1.20	2.50	4.83	6.03	6.93		++	23.25
			0.25	0.27	0.49	0.25	0.19	0.29		
5	Captan	2	0.00	0.00	0.00	0.00	0.00		-	100.00
			0.00	0.00	0.00	0.00	0.00	0.00		
6	COC	2	0.00	0.00	0.00	0.00	0.00		-	100.00
			0.00	0.00	0.00	0.00	0.00	0.00		
7	Control		1.50	3.80	6.67	8.67	9.03		+++	0.00
			0.31	0.48	0.59	0.42	0.07	0.38		
	S.E +		0.08	0.19	0.18	0.14	0.25			
			0.02	0.03	0.01	0.02	0.03			
	C.D. (0.05)		0.23	0.58	0.56	0.42	0.77			
			0.05	0.09	0.03	0.07	0.08			
	CV %		21.82	22.66	13.26	7.97	12.67			
			21.07	28.44	8.68	31.53	44.17			

Note: 1. Figures in bold faces indicate growth rate (mm hr^{-1}) values.
2. Degree of mycelial growth : - NIL, +Poor, ++ Moderate, +++ Good and ++++ Adundant

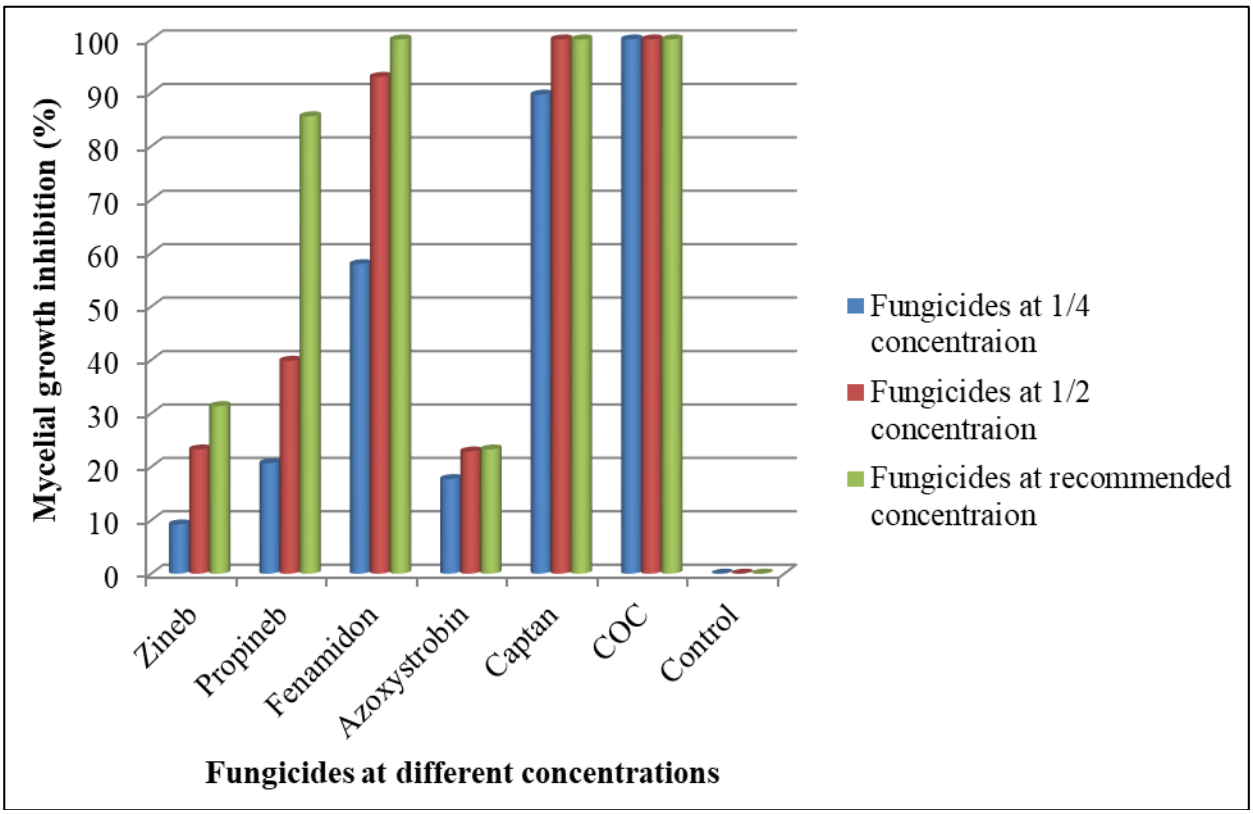

Fig 1: *In vitro* bio-efficacy of fungicides at different concentrations against *C. Gloeosporioides*

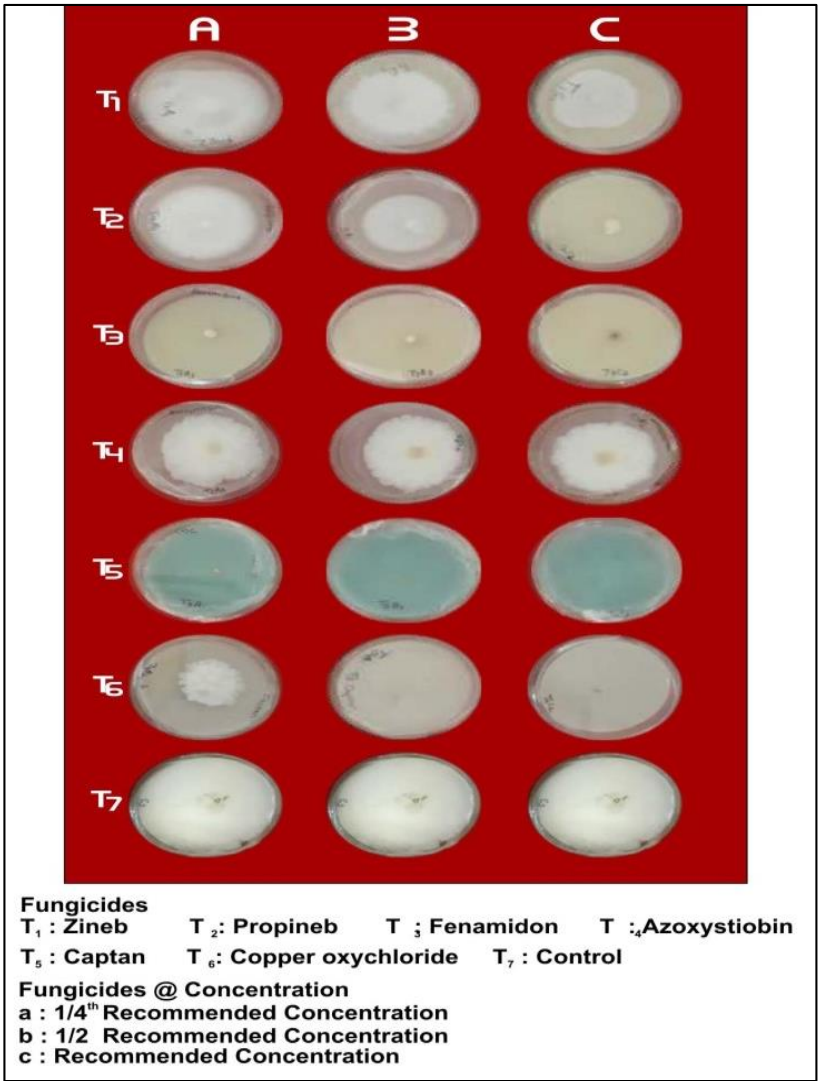

Plate 1: *In vitro* bio-efficacy of fungicides at different concentrations against *C. Gloeosporioides*

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