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Evaluation of fungicides against Gray mould (*Botrytis cinerea* Pers. Fr) and Leaf mould [*Fulvia fulva* (*Cooke*) Cif. (*Cladosporium fulvum Cooke*)] of tomato

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Abstract
Gray mould (*Botrytis cinerea* Pers. Fr) and Leaf mould, [*Fulvia fulva* (Cooke) Cif. (*Cladosporium fulvum* Cooke)] are major disease of tomato causing significant yield losses under protected conditions. Twelve fungicides, including systemic, protectant, coformulations and bicarbonate salts were tested against mycelial growth of *Botrytis cinerea* and *Fulvia fulva,* at 25, 50, and 100 µg ml^{-1} concentration. Among the fungicides tested azoxystrobin + tebuconazole was found to be most effective showing complete inhibition of mycelial growth even at lowest tested concentrations for both the pathogens. Bicarbonate salts of sodium also gave cent percent mycelial growth inhibition of the pathogens

Keywords: Gray mould, Leaf mould, fungicides, bicarbonates, coformulations

Introduction
Gray mould caused by *Botrytis cinerea* Pers. Fr and Leaf mould, caused by *Fulvia fulva* (Cooke) Cif. (*Cladosporium fulvum* Cooke) are the most destructive foliar diseases of tomato grown under humid conditions and is a common problem in greenhouse grown tomatoes. *Botrytis cinerea* infects leaves, stems, flowers and fruits of plants, while *Fulvia fulva* generally infects foliage, fruit infection is rare. These infect either by direct penetration or through wounds caused by cultivation practices. Infestation is stimulated by high humidity, particularly if free moisture is present on the plant surface and low temperatures Shtienberg and Elad, 1997 [13]; Williamson *et al.,* 2007 [17]. Absence of control measures could lead to large portion of leaf damage and significant reduction in tomato fruit yield. (Smith *et al.,* 1969) [15].

Management of these diseases in polyhouse is based on several cultural measures such as good ventilation, temperature control, avoidance of wetting of leaves during irrigation, adequate spacing between plants to manage the canopy and aeration and use of resistant cultivars. However, the fact that the fungus mutates easily limits the use of these cultivars and makes necessary the use of chemicals for the successful management of the disease (Laterrot, 1986) [5]. For chemical control, numerous fungicides with different modes of action are commercially available. Protectant fungicides have been utilized for controlling moulds, but do not offer durable control against the disease. Systemic fungicides often perform better than the protectant fungicides. but intense use of these selects for resistant fungal strains of the pathogens. Leroux and Clerieau, 1985 [6]; Leroux *et al.,* 2002 [7] The use of coformulations with both protectant and systemic modes of action has often been recommended to help manage fungicide resistance development Fujimura, 1993 [3]; Elad *et al*., 1992 [2]. Recently, such coformulations have been formulated which broadens the spectrum of activity to delay selection of resistant fungal populations and aid to optimize efficacy (Roy *et al.,* 2010) [11]. There is considerable interest in the use of bicarbontes for controlling various fungal diseases in plants (Smilanick *et al.,* 2006) [14], as these are inexpensive easy and safe in application, non-hazardous for human and avoids environmental pollution, thus can serve as a better option under restricted conditions. These are found to suppress several fungal diseases (Ziv and Zitter 1992) [19].

The present study was therefore, undertaken to assess the *in vitro* efficacy of coformulations in comparison to solo applications of protectant or systemic fungicides separately and bicarbonates, in controlling gray mould and leaf mould of tomato.

Materials and methods

Isolation of the pathogens

Leaves of tomato exhibiting typical symptoms of Gray mould and leaf mould were collected from polyhouse at Vegetable Research Centre and Precision Farming Development Centre, G.B. Pant University of Agriculture and Technology, Pantnagar. The infested parts were cut aseptically into small pieces and surface sterilized with sodium hypochlorite (1%) for thirty seconds to one minute followed by two to three washing with sterilized distilled water and then placed on sterilized blotter paper to remove excess moisture. Then sterilized pieces were kept aseptically on Petri plates containing potato dextrose agar (PDA). The inoculated Petri plates were incubated at 25±1 °C in BOD incubator. The growing mycelium from the margin of apparently distinct colonies were picked up and subcultured on fresh plates containing medium. The fungus were purified by hyphal tip culture, sub cultured on PDA slants and were preserved in refrigerator at 4 ^{0}C for further use.

In vitro evaluation of fungicides against test pathogens

Efficacy of 12 different fungicides (table:1) representing different modes of action, including systemic, protectant, coformulations and bicarbonate salts against mycelial growth of *Botrytis cinerea* and *Fulvia fulva,* was studied under *in vitro* condition at 25, 50, and 100 µg ml^{-1} concentration by Poisoned Food Technique (Sharvelle, 1961) [12]. Double strength of potato dextrose agar (PDA) was prepared and transferred in 150 ml flask at the rate of 50 ml per flask. A series of double concentration of each test fungicides were prepared in 50 ml of sterile distilled water and added in each flask to get desired concentration, i.e. 25, 50, and 100 µg ml^{-1} of each fungicide. Twenty millilitre of poisoned PDA was poured aseptically in each Petri plate. The Petri plates were inoculated with 5 mm mycelial disc cut with sterilized cork borer from 7 days old culture and transferred aseptically in the centre of each Petri plate. A suitable check was also maintained without adding any fungicide in PDA. Each treatment was replicated five times. Petri plates were incubated at 25 ± 1 °C for 7 days. After 7 days of incubation, observations were recorded by measuring radial growth of the colony at right angles. Mean colony diameter was taken to calculate percent inhibition of radial growth by the formula given by Vincent (1927) [16].

$$\text{Percent growth inhibition} = \frac{\text{Colonyh diameter in check - Colony diameter in treatment}}{\text{Colony diameter in check}} \times 100$$

Table 1: Fungicides used for *in vitro* evaluation against test pathogens

Common Name	Trade Name
Captan	Captaf 50 WP
Chlorothalonil	Kavach 75 WP
Tebuconazole	Folicur 250 EC
Hexaconazole	Contaf 5 SC
Azoxystrobin	Amistar 23 SC
Copper hydroxide	Kocide 77 WP
Potassium bicarbonate	Analytical grade
Sodium bicarbonate	Analytical grade
Fungicidal mixtures	
Azoxystrobin 11% + Tebuconazole 18.3%	Custodia 29.3 SC
Azoxystrobin 4.8% + Chlorothalonil 44%	Amistar opti 560 SC
Captan 70% + Hexaconazole 5%	Taquat 75 WP
Metiram 55% + Pyraclostrobin 5%	Cabriotop 60 WG

Statistical analysis

The data was analysed statistically by Completely Randomized Design (CRD) using statistical software STPR developed by G. B. Pant University of Agriculture and Technology, Pantnagar. Data recorded were compared by the means of critical differences at five per cent level of significance

Results and Discussion

Effect of fungicides on mycelial growth of *Botrytis cinerea* Pers. Fr.

All tested fungicides were found significantly effective in comparison to control in reducing the mycelial growth of the tested pathogen (Table. 2, Fig. 1). At 25 µgml^{-1} concentration azoxystrobin + tebuconazole and azoxystrobin + chlorothalonil were best among all fungicides showing 100 per cent inhibition of mycelial growth of *Botrytis cinerea* and were at par with and captan + hexaconazole (96.67%). Chlorothalonil was found least effective showing 45.19 per cent inhibition at 25 µgml^{-1}. At 50 µgml^{-1} concentration same trend followed with azoxystrobin + tebuconazole, azoxystrobin + chlorothalonil, captan + hexaconazole and sodium bicarbonate showing cent percent inhibition of mycelial growth and were found at par with metiram + pyraclostrobin (96.67%). Least mycelial growth inhibition was recorded in chlorothalonil (50.93%). At 100 µgml^{-1} concentration metiram + pyraclostrobin also showed 100 percent inhibition of mycelial growth along with azoxystrobin + tebuconazole, azoxystrobin + chlorothalonil, captan + hexaconazole and sodium bicarbonate followed by azoxystrobin (96.67%) while chlorothalonil (68.52%) was found least effective among all fungicides.

Effect of fungicides on mycelial growth of *Fulvia fulva* (Cooke) Cif. (*Cladosporium fulvum* Cooke)

All tested fungicides were found significantly superior in reducing the mycelial growth of *Fulvia fulva* over control (Table.3, Fig.2). Among all fungicides azoxystrobin + tebuconazole, sodium bicarbonate and potassium bicarbonate showed 100 percent inhibition of mycelial growth at all tested concentrations *viz.,* 25, 50 and 100 µgml^{-1}. Among other fungicides at 25 µgml^{-1} colony diameter ranged from 13.83 - 54.67 mm being minimum (13.83 mm) in azoxystrobin + chlorothalonil which was at par with captan + hexaconazole (15.67 mm) while maximum (54.67 mm) in captan which was at par with copper hydroxide (50.83mm). At 50 and 100 µgml^{-1} concentrations also cent per cent inhibition was by azoxystrobin + tebuconazole, sodium bicarbonate and potassium bicarbonate, followed by azoxystrobin + chlorothalonil, while least inhibition of mycelial growth was observed in captan (62.04%).

Coformulations gave better control of the pathogen which may be due to additive effect of the protectant and systemic fungicides over solo application of each component fungicide (Rashid *et al*., 2014) [10]. Results of present study also appproved the *in vitro* effectiveness of sodium bicarbonate and potassium bicarbonate in reducing mycelial growth of *B. cinerea,* and *F. fulvia* the causal agents of gray mould, and leaf mould of tomato respectively. Antifungal activity of organic and inorganic salts of bicarbonates have been reported by several researchers for managing plant pathogenic fungi and can be attributed to the presence of bicarbonate compound

(Hang and Woodams, 2003) [4]. Palmer *et al.* (1997) [9] and Nigro *et al.* (2006) [8] found that potassium, and sodium bicarbonates could inhibit the colony growth of *B. cinerea.* Zaker (2014) [18] observed that potassium bicarbonate and sodium bicarbonate were most effective in reducing growth and spore germination of *B. cinerea.* Bombelli and Wright (2006) **[1]** also found that an *in vitro* treatment of tomato fruits with 1% KHCO3 controlled *B. cinerea* by a fungistatic action. From the above study it can be concluded that all tested fungicides have significant adverse effect on mycelial growth of the tested pathogens *viz., Botrytis cinerea* and *Fulvia fulva.* azoxystrobin + tebuconazole was found to be the best treatment showing 100 per cent growth inhibition even at lower dose of 25 µgml^{-1} for both pathogens. At higher concentration (100 µgml^{-1}) azoxystrobin + chlorothalonil, captan + hexaconazole and metiram + pyraclostrobin also showed 100 per cent inhibition of mycelial growth for *Botrytis cinerea.* Among the bicarbonates sodium bicarbonate was cent percent effective for both the fungi and potassium bicarbonate for *Fulvia fulva* and thus can be used for management of the diseases cause by these pathogens in organic farming systems.

Table 2: Effect of fungicides on mycelial growth of *Botrytis cinerea* Pers. Fr.

Treatments	Mycelial growth (mm)			Growth Inhibition (%)		
	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
Captan 50 WP	27.50	23.17	14.50	69.44	74.26	83.89
Chlorothalonil 75 WP	49.33	44.17	28.33	45.19	50.93	68.52
Tebuconazole 25.9 EC	20.00	16.50	13.83	77.78	81.67	84.63
Hexaconazole 5 SC	24.67	14.67	7.17	72.59	83.70	92.04
Azoxystrobin 23 SC	15.67	11.17	3.00	82.59	87.59	96.67
Copper hydroxide 77 WP	20.00	17.67	11.67	77.78	80.37	87.04
Potassium bicarbonate	30.00	22.00	18.00	66.67	75.56	80.00
Sodium bicarbonate	18.00	0.00	0.00	80.00	100.00	100.00
Azoxystrobin 11 + Tebuconazole 18.3 SC	0.00	0.00	0.00	100.00	100.00	100.00
Azoxysrobin 4.8 + Chlorothalonil 44 SC	0.00	0.00	0.00	100.00	100.00	100.00
Captan 70 + Hexaconazole 5 WP	0.00	0.00	0.00	100.00	100.00	100.00
Metiram 55 + Pyraclostrobin 5 WP	16.67	3.00	0.00	81.48	96.67	100.00
Control	90.00	90.00	90.00	0.00	0.00	0.00
CD at 5% Fungicide	2.19					
Concentration	1.05					
Fungicide x Concentration	3.8					
CV	11.9					

Table 3: Effect of fungicides on mycelial growth of *Fulvia fulva* (Cooke) Cif. (*Cladosporium fulvum* Cooke)

Treatments	Mycelial growth (mm)			Growth Inhibition (%)		
	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
Captan 50 WP	54.67	41.50	20.17	39.26	53.89	77.59
Chlorothalonil 75 WP	40.00	32.17	16.67	55.56	64.26	81.48
Tebuconazole 25.9 EC	20.67	15.00	9.67	77.04	83.33	89.26
Hexaconazole 5 SC	36.00	27.00	14.67	60.00	70.00	83.70
Azoxystrobin 23 SC	19.67	14.33	3.00	78.15	84.07	96.67
Copper hydroxide 77 WP	50.83	43.33	31.83	43.52	51.85	64.63
Potassium bicarbonate	0.00	0.00	0.00	100.00	100.00	100.00
Sodium bicarbonate	0.00	0.00	0.00	100.00	100.00	100.00
Azoxystrobin 11 + Tebuconazole 18.3 SC	0.00	0.00	0.00	100.00	100.00	100.00
Azoxysrobin 4.8 + Chlorothalonil 44 SC	13.83	10.00	7.67	84.63	88.89	91.48
Captan 70 + Hexaconazole 5 WP	15.67	12.00	9.33	82.59	86.67	89.63
Metiram 55 + Pyraclostrobin 5 WP	26.33	17.83	12.00	70.74	80.19	86.67
Control	90.00	90.00	90.00	0.00	0.00	0.00
CD at 5% Fungicide	2.27					
Concentration	1.09					
Fungicide x Concentration	3.94					
CV	10.67					

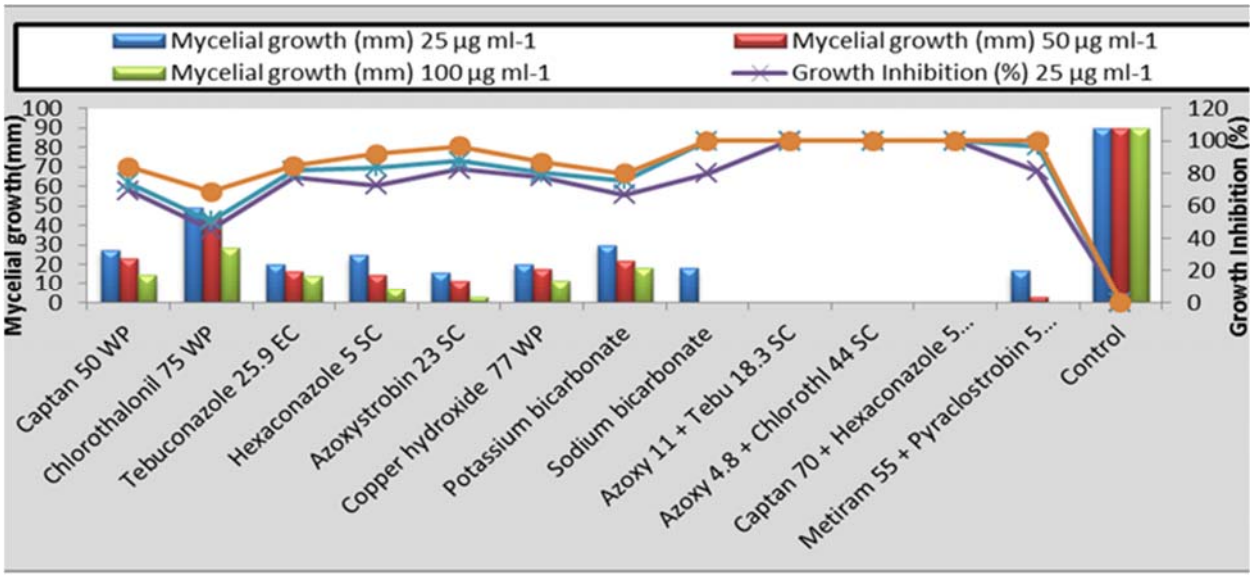

Fig 1: Effect of fungicides on mycelial growth and growth inhibition of *Botrytis cinerea* Pers. Fr.

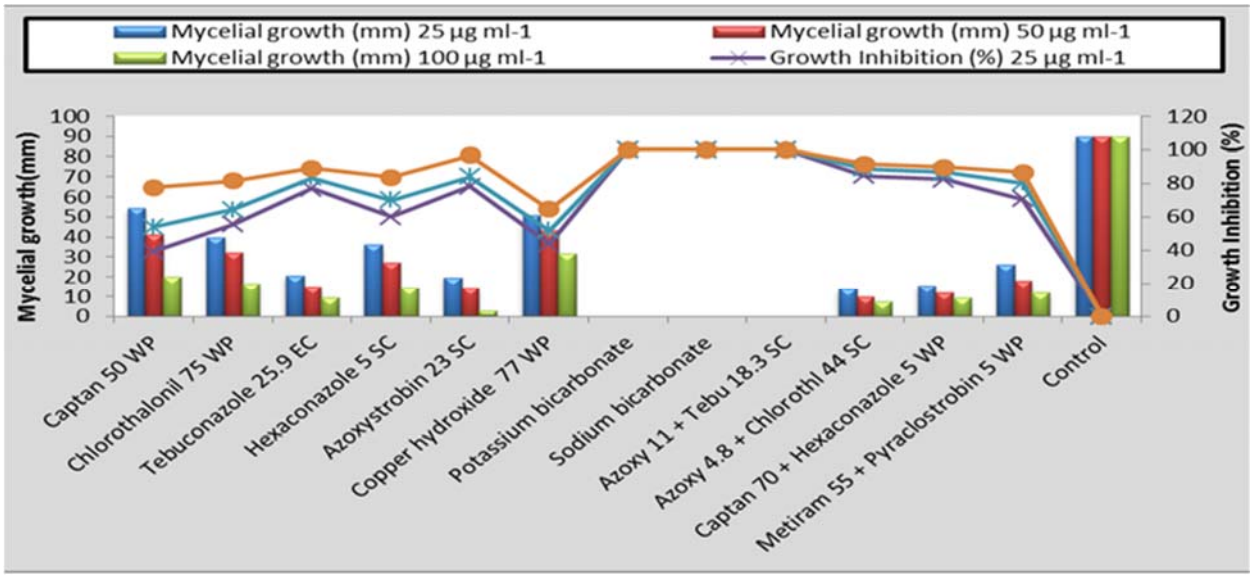

Fig 2: Effect of fungicides on mycelial growth and growth inhibition of *Fulvia fulva* (Cooke) Cif. (*Cladosporium fulvum* Cooke)

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