Growth Potential of Different Fungicide Resistant *Alternaria solani* Isolates of Tomato Leaf Blight Pathogen, under In Vitro, in the Presence of Different Fungicide, Growth Temperature and pH Ion Concentration

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Abstract — Evolution of fungicide resistance in plant pathogens and cross resistance to other fungicides in such pathogens is one of major concerns in sustainable plant disease management. Frequent and unwarranted use of fungicides to control plant diseases not only pollute the earth soil and environment, but also causes the development of fungicide resistance in the plant pathogens, which makes it difficult to manage the plant disease. In India, particularly in western Maharashtra, *Alternaria* leaf blight pathogen of tomato developed resistance and cross resistance to various fungicides. How these fungicide resistant isolates grow in the different fungicidal environment is reported in the present investigation. Eight different fungicide resistant isolates of *Alternaria solani* responsible for causing leaf blight in tomato crop were tested for their growth potential, under in vitro condition, on the potato-dextrose-agar(PDA) growth media amended with different fungicides viz. Dithane M-45, Blitox, Kavach, Ridomil, Nativo, Bavistin, Captaf and Score. Different fungicide resistant isolates showed different pattern of growth i.e. complete inhibition of growth, reduced growth or enhancement of growth on different fungicide amended PDA media. The growth of Dithane M-45 resistant isolate was enhanced by fungicide Kavach and Bavistin while reduced by fungicide Blitox. The growth of this fungicide resistant isolate was completely inhibited by fungicide Ridomil, Nativo, Captaf and Score. The maximum enhancement of growth was for Nativo resistant isolate to Bavistin amended PDA media. Bavistin resistant isolate had decreased growth on all fungicides amended PDA media. The minimum reduction in growth was recorded for Kavach resistant isolate on Dithane M-45 fungicide amended PDA media. The growth rate (cm/day) of these fungicide resistant isolates was maximum at 22°C temperature as compared to 25 and 30°C temperature on the routine PDA growth medium. Captaf resistant isolate produced more growth followed by Nativo, Dithane M-45 and score resistant isolate. The minimum growth was observed for Blitox resistant isolate. As the temperature increases, the growth rate decreased. The growth of fungicide resistant isolates was favored by neutral pH of 7.0 and decreased toward the acidic and alkaline condition. Captaf resistant isolate produced more growth followed by Nativo and score resistant isolates. The minimum growth was produced by Blitox resistant isolate. More growth of fungicide resistant *Alternaria* isolate means production of more inoculum for disease

initiation and spread which is favored at 22 °C temperature and further indicate which fungicide should be used to restrict/manage the growth of particular *Alternaria* fungicide resistant isolate.

Index Terms — tomato, leaf blight, *Alternaria solani*, fungicide resistance, effect of temperature, pH.

I. INTRODUCTION

Tomato (*Solanum lycopersicum*) is an important vegetable fruit crop grown all over the world and is widely succumb to *Alternaria solani* leaf blight fungal pathogen causing losses in the tomato yields to the tune of 78 percent [1]. To manage this important fungal disease, several fungicide molecules are used in different ecological growth environment of the crop [2]-[5]. In western Maharashtra region of India which comprises ten districts, different fungicides like Dithane M-45, Blitox, Kavach, Ridomil, Nativo, Bavistin, captaf and score alone or in alternate/subsequent sprays are used by the tomato growers to manage this disease [6]. Due to frequent and unwarranted use of a single or more than one fungicide in the management of this disease, the fungal pathogen *Alternaria solani* has developed resistant to these fungicides [7]. Several other fungal pathogens are reported to developed fungicidal resistant to different fungicides [8]-[11]. How these fungicide resistant isolates response to the application of other fungicides for their growth in the fungicidal environment is not known. Further how these fungicide resistant isolates response to different temperature and pH regime for their growth is not known and therefore was an important aspect of study so as to know the strategies for the management of fungal growth/inoculum load of these fungicide resistant isolates.

II. MATERIAL AND METHODS

A. Isolation of fungicide resistant *Alternaria solani* isolates from fungicide sprayed tomato crops

In ten district of western Maharashtra, different tomato fields sprayed with different fungicides (Table 1) to control *Alternaria* leaf blight pathogen was selected for the isolation of fungicide resistant *Alternaria* isolates.

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TABLE1: SAMPLING OF DISEASED TOMATO LEAVES FROM INFECTED FIELDS IN DIFFERENT DISTRICTS OF WESTERN MAHARASHTRA SPRAYED WITH FUNGICIDES TO CONTROL *Alternaria* LEAF BLIGHT DISEASE

Sr.No	Trade name of fungicide sprayed (concentration) in tomato fields	Name of district	Number of fields (out of 100)	Nature of fungicide	Technical formulation
1	Dithane M-45 (@ 0.2%)	Ahmadnagar, Jalgaon, Sangli, Dhule, Solapur, Nandurbar, Kolhapur, Pune, Satara, Nashik	75	Contact	Mancozeb 75 Wp
2	Blitox (@ 0.2%)	Jalgaon, Sangli, Dhule, Solapur, Nandurbar, Kolhapur, Satara, Nashik, Ahmadnagar	21	Contact	Copperoxychloride 50% WP
3	Bavistin (@ 0.1 %)	Ahmadnagar, Jalgaon, Sangli, Dhule, Solapur, Nandurbar, Kolhapur, Pune, Satara, Nashik	42	Systemic	Carbendazin 50% WP
4	Captaf (@ 0.2 %)	Ahmadnagar, Nandurbar, Kolhapur, Satara, Pune, Nashik	14	Contact	50% Wp formulation of Captan
5	Score (@ 0.1 %)	Ahmadnagar, jalgaon, Sangli, Kolhapur, Satara, Pune, Nashik	24	Systemic	Trizole fungicide 25% Difenconazole
6	Nativo (@ 0.1 %)	Pune, Dhule, Kolhapur	9	Systemic Broad spectrum With protective & Curative action	Tebuconazole and Trifloxystrobin
7	Ridomil (@ 0.2 %)	Sangli, Dhule, Solapur, Nandurbar, Kolhapur, Pune, Ahmadnagar, Jalgaon	32	Systemic & residual	4% w/w Metalaxyl-M 64% w/w Mancozeb
8	Kavach (@ 0.2 %.)	Sangli, Dhule, Kolhapur, Pune, Nashik, Jalgaon	22	Contact	Chlorothalonil 75% WP

Before isolation of the fungicide resistant *Alternaria* mutant, the efficacy of all the above fungicides at their applied concentrations against *Alternaria* leaf blight pathogen of tomato was tested by poison food technique. The potato dextrose agar (PDA) medium plates amended with respective fungicide were inoculated with the previously isolated culture of *Alternaria solani* from unsprayed tomato crop. The effectiveness of these fungicides was confirmed by inhibition/suppression of fungal growth of the *Alternaria* pathogen. The effective fungicide samples were used in the isolation process of fungicide resistant mutants/isolates.

To isolate the fungicide resistant mutant of *Alternaria solani* formed in tomato crop, the disease sample leaves from known fungicide sprayed crop was collected. The isolation of *Alternaria* disease pathogen was carried out on the PDA medium plates amended with corresponding fungicide at the applied concentration as well as on plain PDA media plates by following the routine isolation technique of fungal pathogen. The inoculated plates were incubated in BOD incubator at 28 ± 2 °C temperature for 7 days. The observations of fungal growth on fungicide amended media and on plain media were recorded and tabulated.

B. Testing of growth potential of different fungicide resistant isolates of *Alternaria solani* in presence of other fungicides

To test the growth potential of different fungicide resistant isolates in presence of other fungicides, the fungal disc (5mm) of fungicide resistant isolate maintained on respective fungicide amended PDA media were used. These discs were placed on PDA amended with different fungicides and the plates were incubated in BOD incubator at 28 ± 2 °C temperature for 7 days and the growth was recorded and tabulated.

C. Testing of growth potential of different fungicide resistant isolates at different growth temperatures

To test the growth potential of different fungicide resistant isolates of *Alternaria solani* at different growth temperatures, three temperatures viz. 22 °C, 25 °C and 30 °C were used. The fungal disc (5mm) of fungicide resistant isolate maintained on respective fungicide containing PDA media was used. These discs were placed on PDA plates amended with the same fungicide and the plates were incubated at 22, 25 and 30 °C temperatures respectively for 7 days and the growth was recorded and tabulated.

D. Testing of growth potential of different fungicide resistant isolates at different pH regime

To test the growth potential of different fungicide resistant isolates of *Alternaria solani* at different pH regime, the neutral pH (7.0), acidic pH (4.0) and alkaline pH (10.0) were used. The fungal disc (5mm) of fungicide resistant isolate maintained on respective fungicide containing PDA media was used. These discs were placed on PDA plates of different pH regime as mention above and amended with fungicide and the plates were incubated at 25°C temperature for 7 days and the growth was recorded and tabulated.

III. RESULT AND DISCUSSION

The growth potential of different fungicide resistant *Alternaria solani* isolates of tomato leaf blight pathogen was studied under in vitro condition in the presence of different fungicides, growth temperatures and pH ion concentration. Eight different fungicide resistant isolates of the pathogen were tested for their growth potential on different fungicide amended PDA growth medium viz. Dithane M-45, Blitox, Kavach, Ridomil, Nativo, Bavistin, Captaf and score fungicide amended PDA media.

Different fungicide resistant isolates showed different pattern of growth i.e. complete inhibition of growth, reduced

growth or enhancement of growth on different fungicide amended PDA media (Table 2).

TABLE 2: GROWTH POTENTIAL OF DIFFERENT FUNGICIDE RESISTANT ISOLATES OF *Alternaria solani* ON OTHER FUNGICIDE AMENDED MEDIA

Sr.No.	<i>Alternaria</i> isolate Resistant to fungicide	Growth (in mm) on other fungicide amended media									
		Dithane M-45	Blitox	Kavach	Ridomil	Nativo	Bavistin	Captaf	Score	SEm±	CD at 5%
1	Dithane M-45	16	9	77.5	0	0	80	0	0	0.469	1.407
2	Blitox	0	50	75	0	0	75	0	0	0.486	1.459
3	Kavach	11	0	75	0	0	80	33	0	0.498	1.494
4	Ridomil	13	0	75	47	0	76	0	0	0.604	1.811
5	Nativo	0	8	82	0	6	86	25	11	0.524	1.571
6	Bavistin	6	18	75	52	35	80	30	18	0.690	2.069
7	Captaf	0	15	74	42	25	75	26	20	0.620	1.858
8	Score	0	0	75	0	0	80	0	25	0.507	1.522

The growth of Dithane M-45 resistant isolate was enhanced by fungicide Kavach and Bavistin while reduced by fungicide Blitox. The maximum enhancement of growth to the tune of 400 percent was observed for Bavistin fungicide while the maximum growth reduction of 43.8 percent was observed for Blitox fungicide. Other fungicides viz. Ridomil, Nativo, Captaf and score completely inhibited the growth of Dithane M-45 resistant isolate. Similar growth pattern was observed for the fungicide resistant isolates of Blitox, Kavach, Ridomil, Nativo, Captaf and score.

The growth of Bavistin resistant isolate was not favored by any fungicide. On the contrary, it was reduced by all the fungicide tested. The maximum reduction in growth up to 92.5 percent was observed for Dithane M-45 fungicide followed by Blitox, score and others.

Fungicide resistant has been reported in apple scab fungus *Venturia inaequalis* and grey mold fungus *Botrytis cinerea* after two years of intensive benzimidazole application in the field [12]. Carbendazim has failed to control wheat scab disease caused by *Fusarium graminearum* in China after 30 years of application [13]. In five of seven potato fields, isolation of *A. solani* collected at the end of the season were significantly less sensitive to chlorothalonil than isolates collected at the beginning of the season [14]. Lydia and Dennis [15] assessed fungicide resistance of two species of *Alternaria* from potato in the columbia basin of Washington in sensitivity to azoxystrobin and boscalid for mycelial growth and spore germination at three fungicide concentration in order to determine the rates which were no longer effective. *Alternaria solani* mycelial growth rates and spore germination on azoxystrobin amended media did not change from 2010 to 2011. A slight decrease in resistance of mycelial growth to azoxystrobin was exhibited among *A. alternata* isolates. No change in sensitivity to azoxystrobin was observed in mycelial growth ratio among *A. alternata* isolates. Resistance to boscalid by *A. solani* increased as indicated by a significant increase in mean mycelial growth ratio from 2010-2011. Avenot and Michailides [9] reported that Boscalid resistant isolates of *Alternaria* leaf blight of pistachio failed to control at a concentration which is effective against naturally sensitive isolates. In India, the tomato growers are using different fungicides to combat the infection of *Alternaria* leaf blight pathogen for more than a decade with less success because of the development of fungicidal resistance in the pathogen as evident from our research. Similarly, the growth potential of different fungicide resistant *Alternaria solani* isolates of the tomato leaf blight pathogen in the presence of different fungicides is

not reported earlier and can be used in the management of fungicide resistance in crop protection.

The growth rate (cm/day) of *Alternaria solani* fungicide resistant isolates was maximum at 22°C temperature as compare to 25 and 30°C temperature (Table 3).

TABLE 3: GROWTH RATE OF FUNGICIDE RESISTANT ISOLATES OF *Alternaria solani* AT DIFFERENT TEMPERATURE

Sr.No.	<i>Alternaria</i> isolates Resistant to fungicide	Growth rate/day (in cm) at a temperature of		
		22 °C	25 °C	30 °C
1	Dithane M-45	0.84	0.74	0.43
2	Blitox	0.60	0.48	0.26
3	Kavach	0.72	0.62	0.38
4	Ridomil	0.62	0.53	0.34
5	Nativo	0.84	0.77	0.58
6	Bavistin	0.82	0.76	0.60
7	Captaf	0.96	0.91	0.64
8	Score	0.84	0.72	0.41
	SEm±	0.012	0.011	0.007
	CD at 5%	0.033	0.034	0.021

Captaf resistant isolate produced more growth followed by Nativo, Dithane M-45 and score resistant isolates. The minimum growth was observed for Blitox resistant isolates. As the temperature increased, the growth rate decreased. The increase in temperature from 22 to 25°C and from 25 to 30°C decreased the growth rate of Dithane M-45 resistant isolates to 11.9 and 41.9 percent respectively. The growth decreased for Blitox resistant isolate was 20.0 and 45.8 percent respectively for these temperatures. For other fungicide resistant isolates, the decreased in growth rate for 22 to 25°C temperature was in the range of 5.2 to 14.5 percent, while for the temperature from 25 to 30°C, it was 18.7 to 43.1 percent.

Jaggal Somapp et.al [16] reported maximum growth and sporulation of tomato *Alternaria* leaf spot pathogen from natural population at 25°C temperature. The conidia of *Alternaria brassicae* grew best at 22 to 25°C [17], while good growth and sporulation of various species of *Alternaria* was reported from temperature of 23 to 28°C [18], [19]. The fungicide resistance isolates of *Alternaria solani* in our studies grew best at 22°C temperature, indicating that fungicide resistant does not alter the temperature requirement for the growth of *Alternaria* isolates. Some of the fungicides in our resistance studies alter the colony morphology colour of the fungus. No such report is available, however the variation in colony character of the isolates was attributed to the genetical attribute of the fungus [20].

The growth rate of fungicide resistant isolates of *Alternaria solani* was influenced by the pH of the growth media (Table 4).

TABLE4: GROWTH RATE OF FUNGICIDE RESISTANT ISOLATES OF *Alternaria solani* AT DIFFERENT pH REGIME

Sr.No.	<i>Alternaria</i> isolates Resistant to fungicide	Growth rate/day (in cm) at pH		
		4	7	10
1	Dithane M-45	0.36	0.73	0.48
2	Blitox	0.36	0.48	0.29
3	Kavach	0.46	0.62	0.50
4	Ridomil	0.41	0.53	0.43
5	Nativo	0.43	0.77	0.43
6	Bavistin	0.59	0.76	0.61
7	Captaf	0.67	0.91	0.60
8	Score	0.35	0.72	0.48
	SEm±	0.008	0.011	0.008
	CD at 5%	0.025	0.034	0.024

At neutral pH of 7.0, all the fungicide resistant isolates produced more growth than at the pH towards the acidic (pH 4.0) and alkaline side (pH 10.0). The Captaf resistant isolates produced more growth followed by Nativo and Bavistin resistant isolates as compare to other fungicide resistant isolates at this pH. The growth rate decreases when the pH of the growth medium was decreased towards acidic side or increased towards alkaline side. The decrease in growth rate for these isolates was in the range of 18.9 to 50.7 percent. Manjunath et.al [21] reported the growth of *Alternaria alternata* was maximum at pH range of 6.0 to 6.5. Gemawat and Ghose [22] reported that pH 6.3 was best for the growth of *Alternaria solani*. Samuel and Govind Swami [23] observed that pH 6.0 was better for *A. carthami*. Gupta and Nikhra [24] reported best pH for growth and sporulation of early blight pathogen of potato was 7.0. Our results also confirmed that all the fungicide resistant isolates produced more growth at 7.0 pH and that fungicide resistance in the pathogen does not alter its pH requirement.

IV. CONCLUSION

The formation of fungicide resistant mutant in the isolates of *Alternaria solani* causing leaf blight of tomato in western Maharashtra region of India poses a serious threat in the management of this important disease on tomato. The development of cross resistant to other fungicides in these fungicide resistant isolates aggravate the problem in control of the disease. Some fungicide increases the growth of these fungicide resistant isolates, thereby producing more inoculum for the spread of the disease. The development of fungicide resistance in *Alternaria solani* of tomato does not alter its requirement of growth temperature and pH regime.

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