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Fungicide resistance in *Alternaria* leaf blight pathogen in tomato crop grown in Satara District

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Abstract
Tomato (*Lycopersicon esculentum* Mill.), an important commercial vegetable of the world, is susceptible to early blight, a devastating fungal (*Alternaria solani* sp & *Alternaria alternata* sp) disease of tomato in India and elsewhere in the world. Currently, sanitation, long crop rotation, and routine application of fungicides are the most common disease control measures. The fungicides used for the management of Alternaria leaf blight in Satara district for the management of this disease were Dithane-M-45, Blitox, Bavistin, Captaf and Score. However, the fungus causing Alternaria leaf blight seems to have developed resistance against Bavistin and Captaf. Therefore, fungicides other than these two fungicide must be recommended on the taluka which have already developed resistance against these fungus.

Keywords: Early blight, Tomato (*Lycopersicon esculantum* L.), *Alternaria solani*, *A. alternata*, fungicide resistance.

Introduction
Tomato (*Lycopersicon esculantum* L.) crop is subjected to several diseases caused by fungi, bacteria, viruses, nematodes and abiotic factors (Blanchard, 1992) [10]. Among the fungal diseases, early blight also known as target spot disease incited by *Alternaria solani* (Ellis and Martin) Jones and Grout, is one of the most catastrophic diseases of the crop in the world as well as in India. Alternaria belonging to sub-division Deutromycotina is a cosmopolitan fungus and is the causal organism of leaf blight diseases in Brassicaceous, Cucurbitaceous and Solanaceous vegetables. Early blight in tomato is the most destructive disease as it accounted for 78 % yield loss at 72 % disease intensity [1]. The disease becomes wide- spread and serious, causing defoliation, drying off of twigs and premature fruit drop causing 50 to 86 % losses in fruit yield (Mathur and Shekhawat, 1986) [8] to the growers when the season begins with abundant moisture or frequent rains followed by warm and dry weather which are unfavorable for the host and help in rapid disease development. The pathogen of the disease has been documented as *A. solani* globally [2–4] as well as in India [5–7]. *Alternaria solani* causes diseases on foliage (early blight), basal stems of seedlings (collar rot), stems of adult plants (stem lesions), and fruits (fruit rot) of tomato (Reni and Roeland, 2006) [9].
Primary methods of controlling early blight include preventing long periods of wetness on the leaf surface, cultural scouting, sanitation, and development of the host plant resistance with the application of fungicides (Namanda *et al*., 2004; Kirk *et al*., 2005 and Kumar and Srivastava, 2013) [12, 13, 14]. Cultivation of resistant varieties is the ultimate control of this disease. Although heritable resistance has been reported for *A. solani* (Christ, 1991; Herriot *et al*., 1986 and Holley *et al*., 1983) [15, 16, 17], the disease is still primarily managed by use of foliar fungicides.
However frequent application of these fungicides over a period of time has led to the development of fungicidal resistance in Alternaria resulting in emergence of fungicidal resistant strains. Tymon and Johnson (2014) [11] reported fungicidal resistance of two fungicides azoxystrobin and boscalid in *A. solani* and *A. alternata*
Though farmers follow different spraying schedules of fungicides to control the disease the incidence of the disease has observed to increase in recent years, inspite of large number of sprays. The probable reason seems to be development of fungicidal resistance in the pathogen which makes the pathogen non sensitive to fungicidal application thereby increase in disease incidence and severity of the disease in Western Maharashtra.

Material and Methods
Disease samples showing typical symptoms of Alternaria leaf spot of tomato were collected

from five taluka of Satara district viz., Phaltan, Man, Satara, Karad and Patan from the tomato fields where fungicidal spray of different fungicides was done. The samples were collected randomly from 10 farmer's field from each district and the collected samples were preserved in paper bags for further isolation.

Isolation of fungicide resistant Alternaria from disease samples

The disease sample was subjected for the isolation of Alternaria pathogen. The Alternaria fungi was isolated from collected disease samples on the medium containing respective fungicides and its concentration were as used in spray schedule by the respective farmer, as listed in Table 1; using poison food technique (Nene and Thapliyal, 2000). The plates were incubated at 30 °C and the observations of fungal growth colonies were taken after 9th days. The Alternaria isolates, appearing on the respective poison food plants were regarded as fungicidal resistance isolates which were then maintained on PDA slants of respective fungicide at given concentration.

Table 1: Fungicidal spray applied by the farmers in the tomato field at Satara District of Western Maharashtra

Sl. No.	Trade name	Active Ingredient (s)	Concentration
1.	Dithane-M-45	Mancozeb	0.2 %
2.	Bavistin	Carbendazim	0.2 %
3.	Blitox	Copper oxy chloride (COC)	0.2 %
4.	Captaf	Captan	0.2 %
5.	Score	Di Fenocunazole	0.2 %

Result and Discussion

The disease samples infected with *Alternaria* leaf blight pathogen in tomato crop was collected from at least five taluka of Satara district namely Phaltan, Man, Satara, Karad and Patan. The infection of the pathogen was assessed for the fungicide resistance in Satana district and is presented in Table 2. In Phaltan taluka the farmers commonly used Dithane-M-45, Bavistin, Blitox, and Captaf. The assessment report of fungicidal resistance in Phaltan taluka showed that the *Alternaria* leaf blight pathogen develop resistance to fungicide Bavistin, where as for other fungicides, the pathogen was still found to be susceptible. In Man Taluka, the fungicide Blitox, Score and Captaf are commonly used. The leaf blight pathogen in this taluka had developed resistance to Captaf. However, it was still susceptible to the fungicide Blitox and Score. In Satara taluka, the fungicide Dithane-M-45, Blitox, Captaf and Bavistin are mostly used. The assessment result showed that in this taluka the pathogen had developed resistance to the fungicide Captaf while it was still susceptible to other applied fungicide. In Karad taluka, the commonly used fungicides are Dithane-M-45 and Captaf and the pathogen had developed resistance against the fungicide Captaf. In Patan taluka, the commonly used fungicides are Dithane-M-45 and Captaf and the pathogen had developed resistance to fungicide Captaf. These results indicates that in Satara district the *Alternaria* leaf blight pathogen had developed resistance to the Bavistin and Captaf fungicide and therefore their use in these taluka should be restricted. The growth rate of fungicide resistant isolate was slow as compared to the non-fungicide resistant isolate. The growth deficit in the presence of fungicide Bavistin was upto 4 cm at 7th days whereas it ranges from 1.6 - 3.6 cm in case of fungicide Captaf, indicating that in the presence of fungicide the growth of the pathogen was very slow as compared to the absence of fungicidal spray on crop. The results also indicated that the Bavistin resistance developed at Gunaware produces the fungal growth at par with the mycelial growth produced in the absence of fungicide. Many other researchers also have similar reports. Mrunalini *et al.*, (2015) [18] also have reported the development of carbendazim resistance in *Alternaria dauci* causing leaf blight of carrot. Murugan *et al.*, 2016 [19] also has reported that the Characterization based on culture colour and conidial dimensions (conidial length and breadth, and beak length) indicated that no distinguished variation of the isolates was observed on the colour parameters. Although, a wide variation was observed in the length of conidia and beak, no significant difference was observed in conidial breadth. The Pathogenicity test on tomato revealed that both *A. alternata* and *A. solani* isolates were of virulent category indicating that former is also an incitant of early blight in northern India. However, in our findings the fungal resistant isolate produced deeper colour of mycelia growth. The Captaf resistant isolate had whitish to light gray colour growth.

Table 2: Development of fungicide resistance in *Alternaria* leaf blight pathogen in tomato crop fields in Satara district

Sr. No.	Village, Tal.	Name of farmers	Sprays applied by farmer (@ 0.2 %)	Isolation and growth of *Alternaria* on respective fungicide containing PDA	Diameter of fungal growth (cm) on fungicide containing media (at 7 days)	Diameter (cm) of fungal growth on PDA media without fungicide (at 7 days)	Colony morphology (colour of fungal isolate)
1	Saspade, Tal. Satara	Shri. Manikrao Tukaram Pawar	Dithane-M-45	X	0	4	-
			Blitox	X	0	2	-
2	Saspade, Tal. Satara	Shri. Pawar Sandeep Hindurao	Dithane-M-45	X	0	2.9	-
			Bavistin	√	1.1	5.1	Gray colour
3	Saspade, Tal. Satara	Shri. Lade Suresh Baburao	Dithane-M-45	X	0	2	
			Bavistin	X	0	4.4	Whitish colour
			Captaf	√	1.9	3.5	Whitish colour
4	Guaware, Tal. Phaltan	Shri. Vishnu Appa Adhav	Bavistin	√	2.9	3	Gray colour
			Dithane-M-45	X	0	3.5	-
5	Gunaware, Tal. Phaltan	Shri. Balaso Jagannath Pise	Dithane-M-45	X	0	5	-
			Bavistin	X	0	3	-
6	Gunaware, Tal. Phaltan	Shri. Anil Ramchandra Khomne	Blitox	X	0	2.5	-
			Captaf	X	0	2.5	-
7	Andhrud, Tal. Man	Shri. Rajendra Sonabapu Pise	Blitox	X	0	1.9	-
			Captaf	X	0	4	-
8	Duberewadi, Tal. Man	Shri. Mahadev Bapu Shingade	Score	X	0	2.3	-
			Captaf	√	1.4	4.5	Light gray
9	Saspade, Tal. Patan	Shri. Devraj Krushna Pawar	Dithane-M-45	X	0	3.5	-
			Captaf	√	1.5	4.5	Dull whitish colour
10	Karve, Tal. Karad	Shri. Santosh Bapu Pawar	Dithane-M-45	X	0	3	
			Captaf	√	1.3	4.9	Whitish colour

X = No Growth √ = Growth

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