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# Development of cross resistance in fungicide resistant isolates of Alternaria leaf blight pathogen of tomato in western Maharashtra

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## Abstract
The fungicide resistance isolates in Alternaria leaf blight pathogen on tomato crop were isolated from 10 districts of Maharashtra against eight fungicides namely Dithane-M-45, Blitox, Kavach, Bavistin, Captaf, Nativo, Ridomil and Score. The percentage disease intensity (PDI) of after 7th day of inoculation shows that the Kavach and Nativo resistant isolate induced the maximum PDI of 21.33 and 20.44 per cent. The minimum PDI was induced by Dithane-M-45 resistant isolates with the PDI of 14.67 per cent which was followed by Blitox with PDI of 16.89 per cent. The Bavistin resistant isolate had developed cross resistance isolate to all seven other above fungicide applied in the tomato field. The Captaf resistant isolate had shown cross resistant to all other fungicide accept Dithane-M-45.The development of fungicide resistance and cross resistance to other fungicides has a serious consequence in the management of this disease in Western Maharashtra.

**Keywords:** Alternaria leaf blight, cross resistance, fungicide resistance, tomato

## Introduction
*Alternaria solani* (Ellis & Martin) Jones & Grout is a soil inhabiting, air-borne fungal pathogen responsible of tomato leaf blight, and collar and fruit rot (Datar and Mayee 1981) [6]. It is the second most important disease of tomato after leaf curl virus causing severe loss everyyear (Somappa, 2013) [21]. It causes reduction in crop quantity and quality. The loss due to early blight in unsprayed fields vary enormously from 5 – 78% (Kemmitt, 2002) [11]. Borkar (2007) [1] reported the yield losses due to Alternaria and bacterial canker complex up to Rs. 15 000/ha. Ruchi and Sharma (2004) [18] reported the incidence of fruit rot (*Alternaria* spp.) of tomato in the range 4.81-11.75%. Current strategies to control early blight disease consist of preventing wetness on leaf surface during long periods of time, development of host plant resistance and application of fungicides. Different Synthetic chemical fungicides including Ridomil gold plus, Mancozeb, copper oxychloride, carbendazim, captafol and benomyl have been used today to control tomato early blight (Chohan *et al.* 2015 [4]; Saharan *et al.* 2015) [19]. History of perspectives of fungicide resistance can be considered to start with the work of Charles Darwin, who pointed out in The Origin of Species (Darwin, 1859) [5] the capacity for variation that exist in living organisms and role of the selection of variants as a driving force in evolution. The change of a pathogen population from sensitive to resistant, through the selection of resistant mutants in response to external changes (i.e. fungicide application), is clearly an evolution process, a very rapid one that greatly affects the survival of the fungus (‘The survival of the fittest’). The evolution of fungicide resistance can be divided into an emergence phase and a selection phase (van den Bosch and Gilligan, 2008 [23]; Milgroom, 1990 [16]; van den Bosch *et al*., 2011) [24]. Culturing of sensitive isolate of *Alternaria alternata* on carbendazim, continuously for eight successive passages showed significantly increased resistance (Khandare, 2014) [12]. Malandrakis *et al*., (2015) [13] first time reported that *A. alternata* isolates were specifically resistant to the multi-site inhibitor mancozeb. Isolates resistant to mancozeb were more aggressive while those with reduced sensitivity to both fludioxonil and iprodione produced significantly less conidia than sensitive ones. Fungal isolates that are resistant to one fungicide are often also resistant to other closely-related fungicides, even when they have not been exposed to these other fungicides, because these fungicides all have similar mode of action. This phenomenon is called cross resistance. Fungicide Group Codes designating chemical groups were developed to facilitate managing resistance by the Fungicide Resistance Action Committee (FRAC) (Brent, 2012) [2]. These codes are usually on the front of labels or in the resistance management section.

Fungicides with the same Group Code have similar mode of action and therefore likely will exhibit cross resistance (McGrath, 2015) [15]. Though farmers follow different spraying schedules of fungicides to control the disease, the incidence of the disease has observed to increase in recent years, in spite of large number of sprays. The larger the pathogen population exposed to an at-risk fungicide, the greater the chance for a resistant strain to develop. 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. Therefore, the study on the developed fungicide resistance in *Alternaria* in tomato crop and their management was carried out.

**Material and Methods**

Disease samples showing typical symptoms of Alternaria leaf spot of tomato were collected from 10 different districts of Western Maharashtra viz., Ahmednagar, Nasik, Pune, Dhule, Nandurbar, Sangli, Satara, Solapur, Kolhapur and Jalgaon, 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) [17]. The plates were incubated at 30 °C and the observations of fungal growth colonies were taken after 9thdays. 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. One each of the most Fungicide resistant isolate within the fungicide screened was chosen for cross resistance study.

**Pathogenicity studies of the fungicide resistant *Alternaria* isolates under glass house conditions**

Pure cultures of *Alternaria* isolates were macerated in a sterile blender and filtered through double layers of cheese cloth to remove large particles. Healthy 22 days old tomato leaves (which were previously injured by rubbing of carborundum powder) were sprayed with the Fungicide resistant isolates filtrate using an atomizer; control plants were sprayed with distilled water. A clear plastic sheet was used to separate the treatments during spraying. Sprayed plants were maintained at 25±3 °C greenhouse. Plants were sprayed to run-off and leaves were allowed to dry before incubation at 100% Relative Humidity in a moist chamber for 48 hours at room temperature (23−25 °C). The disease developed in pathogenicity test was recorded after a weeks on inoculated plants. Plants were arranged in a complete randomized block design and maintained at 25±3 °C in a greenhouse (Margaret *et al.* 2011) [14].

**Tomato leaf damage by *A. solani***

Five plants were selected randomly in each plot and observation on severity of the disease on the foliage was recorded using 0-5 scale (Horsefall and Barette 1945 [10], Sahu *et al.* 2013) [20] (Table 1) and percent disease index (PDI) was worked out usingformula of Wheeler (1969) [26] as given here:

$$PDI = \frac{\text{Sum of all the numerical diseaserating} \times 100}{\text{Total No. of leavesobserved} \times \text{Maximumdisease rating (5)}}$$

**Table 1:** Disease rating scale for the assessment of early blight of tomato

| Scale | Description of the symptom |
|---|---|
| 0 | Leaves free from infection |
| 1 | Small irregular spots covering <5% leaf area |
| 2 | Small irregular brown spots with concentric rings covering 5.1-10% leaf area |
| 3 | Lesions enlarging, irregular brown with concentric rings covering 10.1-25% leaf area |
| 4 | Lesions coalease to form irregular and appears as a typical blight symptom covering 25.1-50% leaf area |
| 5 | Lesions coalease to form irregular and appears as a typical blight symptom covering >50% leaf area |

**Studies on cross resistance of the fungicide resistant Alternaria isolates**

The respective/specific fungicide resistant isolate (of Alternaria) was grown on different fungicide to check their cross resistant to other fungicide by using poison food technique. The observations were recorded after 9days of incubation at 30 °C (Malandrakis *et al.,* 2015) [13]. The percentage Disease control

**Result and Discussion**

In survey of ten districts of Western Maharashtra viz., Ahmednagar, Nasik, Pune, Dhule, Nandurbar, Sangli, Satara, Solapur, Kolhapur and Jalgaon; it was found that eight fungicide viz., Dithane-M-45, Bavistin, Blitox, Captaf, Score, Nativo, Kavach and Ridomil (Table 2) were frequently used by the farmer for the management of Alternaria leaf blight pathogen of tomato and have developed resistance against the fungicides. The Fungicide resistant isolates were subjected to check its pathogenicity on tomato 22 days old tomato plant after isolation from the poison food plates. The symptom induced by the fungicide resistance isolate varies upon the 7thday of inoculation. Dithane-M-45 and Ridomil resistant isolate induces a symptom which were slight yellow halo with darken necrosis around the injured leaf portion. The Blitox and Score resistant isolate induces few visible symptoms with very little yellow halo appearance and darken necrosis around the inoculation leaf portion. The Nativo resistant isolate developed a dark spots around the inoculated area but there were no halo appearance around the injured area. The Kavach, Bavistin and Captaf resistant isolate induces prominent symptom which includes yellow halo around the inoculated portion and surrounding portions. The control uninoculated produces a hypersensitive due to leaf injury and necrosis occurs but no yellow halo appearance or dark spots developed at the injured site. The percentage disease intensity (PDI) of after 7thday of inoculation (Table 2) shows that the Kavach and Nativo resistant isolate induced the maximum PDI of 21.33 and 20.44 per cent. The Nativo, Ridomil and Captaf resistant isolates showed PDI of 20.44, 20.00, and 19.11 per cent, respectively, which were statistically at par with each. The PDI for Ridomil, Captain and Bavistin were statistically at par. The Bavistin and Score resistant isolate showed PDI of 19.11 and 18.22 per cent which were also statistically at par. The minimum PDI was induced by Dithane-M-45 resistant isolates with the PDI of 14.67 per cent which was followed by Blitox with PDI of 16.89 per cent. The variation in pathogenicity of *Alternaria* isolates on tomato plant under greenhouse conditions was also been reported by several researches (Tong *et al*., 1994 [22]; Castro *et al.,* 2000 [3]; Verma *et al.,* 2007) [25].

The eight fungicide resistant isolate were tested against each other to record the development of cross resistance to other fungicide within these fungicide resistant isolate (Table 4). The result indicates that the Dithane-M-45 resistant isolate had developed cross resistance to Blitox, Kavach and Bavistin. The Blitox resistant isolate had developed cross resistant to Kavach and Bavistin. The Kavach resistant isolate had developed cross resistant to Mancozeb, Captaf and Bavistin. The Ridomil resistant isolate had developed cross resistance to Dithane-M-45, Kavach and Bavistin. The Nativo resistant isolate had developed cross resistance isolate to Blitox, Captaf, score, Kavach and Bavistin. The Bavistin resistant isolate had developed cross resistance isolate to all seven other above fungicide applied in the tomato field. The Captaf resistant isolate had shown cross resistant to all other fungicide accept Dithane-M-45. The Score resistant isolate had developed cross resistance to Kavach and Bavistin.

The study on percent disease control of the fungicide resistant isolate shows that the fungicide Blitox, Nativo, Captaf, and Score show complete disease control on the Dithane-M-45 Resistant Alternaria isolates; followed by Dithane-M-45 (82.22%), Kavach (87.78%), Ridomil (85.56%), and Bavistin (93.33%) (Table 5). The fungicide Kavach, Ridomil and Score showed complete control on the Blitox Resistant Alternaria isolates; followed by Nativo(91.11%), Dithane -M-45(90%), Captaf (83.33%), Bavistin (80%) and Blitox(44.44%). The fungicide Captaf showed the highest percent disease control of 17.78% on the Kavach Resistant Alternaria isolates; followed by Blitox (16.67%), Kavach (16.67%), Ridomil (16.67%), Bavistin (16.67%), Score(16.67%), Dithane-M-45 (13.89%) and Nativo (8.89%). The fungicide Dithane - M-45, Blitox, Score, Kavach and Nativo showed complete control on the Ridomil Resistant Alternaria isolates; followed by Ridomil (47.78%), Bavistin (42.22%) and Captaf (53.33%). The fungicide Dithane -M-45, Blitox, Kavach, Ridomil and Score showed complete control on the Nativo Resistant Alternaria isolates; followed by Nativo (93.33%), Captaf (72.22%) and Bavistin (61.11%). The fungicide Score and Blitox showed the highest percent disease control of 16.67% on the Bavistin Resistant Alternaria isolates; followed by Ridomil (15.56%), Dithane -M-45 (11.11%), Kavach (11.11%), Nativo (4.44%), Bavistin (11.11%) and Captaf (11.11%). The fungicide Dithane -M-45, Ridomil, Blitox and Score showed complete control on the Captaf resistant Alternaria isolates; followed by Nativo (72.22%), Captaf (71.11%), Bavistin (66.67%) and Kavach (63.33%). The fungicide Dithane -M-45, Blitox, Kavach, Ridomil showed complete control on the Score Resistant Alternaria isolates; followed by Nativo (87.78%), Bavistin (80%), Captaf (77.78%), Score(72.22%). Hobbelen *et al*., (2014) studied the usefulness of mixing two fungicides of differing modes of action for delaying the emergence of resistance. The results suggest that it is unlikely that a resistant strain will already have emerged when a fungicide with a new mode of action is introduced. Several workers have reported the fungicidal activity and cross resistance of fungicides against Alternaria leaf blight pathogen. Malandrakis *et al.,* (2015) [13] reported that a number of *A. alternata* isolates were specifically resistant to the multi-site inhibitor Mancozeb. Fadl *et al*., (1985) [7] in laboratory tests with 3 fungicides observed that Ridomil MZ (Metalaxyl) was most inhibitory to linear growth at low concentration. Fairchild *et al*., (2013) [8] studied on early blight, caused by the fungus *Alternaria solani* and brown leaf spot, caused by *Alternaria alternata*, are important diseases of potato crops in Idaho and showed that 57% of the isolates were resistant to Boscalid as well as an average of 63% of the isolates being resistant to the Strobilurin fungicides. Seven and 15% of isolates were resistant to Penthiopyrad (an SDH inhibitor), and Pyrimethanil (a methionine biosynthesis inhibitor), respectively. However, none of the isolates were resistant to fluopyram (an SDH inhibitor) or a mixture of fluopyram and pyrimethanil. It was further reported that the cross resistance developed in Alternaria spp. to some of the new SDH inhibitors like Penthiopyrad, others such as Fluopyram are still showing limited to no resistance development in Alternaria spp. in Idaho. The result apparently shows that the Dithane-M-45 Resistant isolates could be managed by the fungicide Nativo, Captaf, Blitox and Score. The Blitox Resistant isolates could be managed by the fungicide Kavach, Ridomil and Score. The Ridomil Resistant isolates could be managed by the fungicide Dithane -M-45, Blitox, Score, Kavach and Nativo. The Nativo Resistant isolates could be managed by the fungicide Dithane -M-45, Blitox, Kavach, Ridomil and Score. The Captaf Resistant isolates could be managed by the fungicide Dithane -M-45, Ridomil, Blitox and Score. The Score Resistant isolates could be managed by the fungicide Dithane -M-45, Blitox, Kavach and Ridomil. However, Bavistin and Kavach Resistant isolates could not be managed completely by any of the eight fungicide (Table 1). Development of fungicidal resistance and further cross resistance to other fungicides seems to be the reason that why the Alternaria blight in tomato has become unmanageable disease in Western Maharashtra in recent years.

**Table 2:** List of fungicide used for isolation of fungicide resistant Alternaria

| 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 | Captaf | 0.2% |
| 5 | Score | Di Fenocunazole | 0.2% |
| 6 | Nativo | Trifloxystrobin 25% w/w + tebuconazole 50% w/w | 0.2% |
| 7 | Kavach | Chlorothalonil | 0.2% |
| 8 | Chipco /Ridomil | Ipirodione | 0.2% |

**Table 3:** Percentage Disease Intensity of the Fungicide resistant isolates of Alternaria after 14 days after inoculation.

| Sl. No. | Isolate Resistant to fungicide | Percentage Disease Intensity |
|---|---|---|
| 1 | Dithane-M-45 | 21.78 |
| 2 | Blitox | 26.67 |
| 3 | Kavach | 32.44 |
| 4 | Ridomil | 28.00 |
| 5 | Nativo | 24.00 |
| 6 | Bavistin | 33.33 |
| 7 | Captaf | 29.78 |
| 8 | Score | 28.89 |
| 9 | S.E.m± | 0.474 |
| 10 | CD at 5% | 0.142 |

**Table 4:** Determination of cross fungicide resistant isolates of Alternaria

| Sl. No. | Resistant isolates | Resistance observed from Poison Food Plates | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dithane-M-45 | Blitox | Kavach | Ridomil | Nativo | Bavistin | Captaf | Score |
| 1. | Dithane-M-45 | Y | N | Y | Y | N | Y | N | N |
| 2. | Blitox | Y | Y | N | N | Y | Y | Y | N |
| 3. | Kavach | Y | Y | Y | Y | Y | Y | Y | Y |
| 4. | Ridomil | N | N | N | Y | N | Y | Y | N |
| 5. | Nativo | N | N | N | N | Y | Y | Y | N |
| 6. | Bavistin | Y | Y | Y | Y | Y | Y | Y | Y |
| 7. | Captaf | N | N | Y | N | Y | Y | Y | N |
| 8. | Score | N | N | N | N | Y | Y | Y | Y |

**Table 5**. Growth (in mm) of Fungicide resistant isolates of Alternaria in presence of other cross resistant developed fungicides

| Sl. No. | Resistant isolate | Growth (in mm) of *Alternaria* resistant isolates in poison food plates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Dithane -M-45 | Blitox | Kavach | Ridomil | Nativo | Bavistin | Captaf | Score | Control |
| 1 | Dithane -M-45 | 16.00 | 0.00 | 11.00 | 13.00 | 0.00 | 6.00 | 0.00 | 0.00 | 90.00 |
| | | (82.22%) | (100%) | (87.78%) | (85.56%) | (100%) | (93.33%) | (100%) | (100%) | (0%) |
| 2 | Blitox | 9.00 | 50.00 | 0.00 | 0.00 | 8.00 | 18.00 | 15.00 | 0.00 | 90.00 |
| | | (90%) | (44.44%) | (100%) | (100%) | (91.11%) | (80%) | (83.33%) | (100%) | (0%) |
| 3 | Kavach | 77.50 | 75.00 | 75.00 | 75.00 | 82.00 | 75.00 | 74.00 | 75.00 | 90.00 |
| | | (13.89%) | (16.67%) | (16.67%) | (16.67%) | (8.89%) | (16.67%) | (17.78%) | (16.67%) | (0%) |
| 4 | Ridomil | 0.00 | 0.00 | 0.00 | 47.00 | 0.00 | 52.00 | 42.00 | 0.00 | 90.00 |
| | | (100%) | (100%) | (100%) | (47.78%) | (100%) | (42.22%) | (53.33%) | (100%) | (0%) |
| 5 | Nativo | 0.00 | 0.00 | 0.00 | 0.00 | 6.00 | 35.00 | 25.00 | 0.00 | 90.00 |
| | | (100%) | (100%) | (100%) | (100%) | (93.33%) | (61.11%) | (72.22%) | (100%) | (0%) |
| 6 | Bavistin | 80.00 | 75.00 | 80.00 | 76.00 | 86.00 | 80.00 | 75.00 | 80.00 | 90.00 |
| | | (11.11%) | (16.67%) | (11.11%) | (15.56%) | (4.44%) | (11.11%) | (16.67%) | (11.11%) | (0%) |
| 7 | Captaf | 0.00 | 0.00 | 33.00 | 0.00 | 25.00 | 30.00 | 26.00 | 0.00 | 90.00 |
| | | (100%) | (100%) | (63.33%) | (100%) | (72.22%) | (66.67%) | (71.11%) | (100%) | (0%) |
| 8 | Score | 0.00 | 0.00 | 0.00 | 0.00 | 11.00 | 18.00 | 20.00 | 25.00 | 90.00 |
| | | (100%) | (100%) | (100%) | (100%) | (87.78%) | (80%) | (77.78%) | (72.22%) | (0%) |
| S.Em.± | | 0.469 | 0.486 | 0.498 | 0.604 | 0.524 | 0.69 | 0.62 | 0.507 | |
| CD at 5% | | 1.407 | 1.459 | 1.494 | 1.811 | 1.571 | 2.069 | 1.858 | 1.522 | |

Note: Values in brackets are the % of disease control

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