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Studies on Alternaria Leaf and Fruit Spot of Pomegranate caused by *Alternaria alternata* (Fr.) Keissler

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ABSTRACT

Pomegranate (*Punica granatum* L.) is regarded as important functional food because of its nutritional importance. In view of its health benefits, consumption of pomegranate fruit is also augmented as a result there is increase in the area of pomegranate cultivation. Pomegranate cultivation is challenged by many biotic and abiotic stresses. Fruit spots or rotting caused by fungi are becoming major constraints, affect the quantity and quantity of the produce. Roving survey was conducted in the year 2017-18 to know the severity of the leaf and fruit spot diseases of pomegranate in Kachchh district of Gujarat. Total five talukas were surveyed. Among the fruit spot causing fungi, *Alternaria alternata* was found as one of the foremost reason for fruit spotting. Among the five talukas surveyed Bhachau taluka recorded the maximum disease severity on both leaf and fruit. Further *in vitro* evaluation studies showed that the maximum mycelial growth can be inhibited by using bioagent *Trichoderma viride* and botanical Nimbecidine 1500 ppm. Among different contact, systemic and combiproducts fungicides, Hexaconazole 5% EC, Propiconazole 25% EC, Difenconazole 25% EC and Tebuconazole 250% EC recorded cent per cent inhibition at all the concentrations tested. Fungicide products in combination with triazole fungicide recorded the maximum inhibition as compared to other combiproduct fungicides. Bioagents, botanicals and traizole fungicides showed maximum inhibition of mycelial growth of *A. alternata* can be further evaluated under field condition for effective management of the disease.

Keywords Survey, Isolation, Fungicides, Bioagents, Botanicals.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is regarded as important functional food because of its nutracitical importance. It is adapted to all types of land and environmental condition. Pomegranate is native fruit crop of Turkey (Ercisli *et al.* 2007). It is cultivated commercially in India, Turkey, Iran, Tunisia, Spain, Afghanistan, Morocco, China and Japan. Among India's various pomegranate growing states, Maharashtra is the leading producer followed by Gujarat, Karnataka, Andhra Pradesh, Madhya Pradesh and Rajasthan (Anonymous 2019). Pomegranate fruits are rich source of vitamins and minerals, maintain a

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balanced diet. Seeds contain low quantity of fat with high amount of carbohydrate and protein. Potassium present in the fruit maintains blood pressure, muscle synthesis, antioxidants like anthocyanins and tannins, minimizes the cellular damage from oxidation and prevents the build up of cholesterol in arteries (Qamar *et al.* 2018, Kandylis and Evangelos 2020). All the parts of the plants are utilized in industrial sector.

Considering increasing awareness of health and medicinal benefits of pomegranate, consumption is also increased as a result there is increase in the area of pomegranate cultivation. Now days the cultivation of pomegranate is challenged by many biotic and abiotic stresses. Biotics tresses like disease and pest damage are major constraints for production of quality fruits. The diseases caused by fungi, bacteria and nematodes take a heavy toll on the crop. Fruit spots or rotting caused by fungi are becoming major constraints, affect the quantity and quantity of the produce. Spots on the fruits reduce the market value.

In India, Gujarat is the second largest pomegranate producing state and Kachchh district of Gujarat state is second leading pomegranate producing district because the plant is drought tolerant, winter hardy and can thrive well under desert condition. Hence the study has been conducted in the Kachchh district in the year from 2017-2019, to know the occurrence and prevalence of pomegranate leaf and fruit spots and their management studies in *in vitro* condition. Among the various leaf/fruit spots the alternaria leaf/fruit spot was found as major disease, causing severe yield losses. Initial symptoms of the disease starts on the leaf with small brown to black color spots with or without concentric rings, these spots increase in size and causes chlorosis and abscission of leaves (David *et al.* 2015). During the survey symptoms were also observed on the flowers, heavily infected flowers were drop downed. The infection further develops on the fruit with black color spots, these spots cover the larger area and affect the market value of the fruits. Infection on the fruit was confined to rind and edible arils were unaffected. But David *et al.* (2015) reported that infection chain of *Alternaria alternata* continues, spore penetrates the pistil of open flowers and grows into the tunnel causing rot of arils leading heart rot of pomegranate. By considering the severity of the disease in detail investigation was undertaken to know the severity of the disease in the major pomegranate growing areas of Kachchh, symptomatology of the disease and identification of the best bioagent, botanical and chemical for the management of *Alternaria alternata* under *in vitro* condition. The best components can be further tested under field condition.

MATERIALS AND METHODS

The present work on studies on alternaria leaf and fruit spot of pomegranate caused by *Alternaria alternata* (Fr.) Keissler was carried out during the year 2017-19 at Regional Research station, S. D. Agricultural University, Bhachau, Kachchh, Gujarat.

Survey : An extensive roving survey was conducted in major pomegranate growing talukas viz., Bhachau, Anjar, Bhuj, Mundra and Nakatrana, of Kachchh district of Gujarat. Severity was recorded by following 0 to 5 scale on leaf and on fruit during fruit development stage to maturity stage.

Disease severity scale for pomegranate diseases.

Grade	Per cent area of infection		Reaction
	On fruit	On leaf	
0	No infection	No infection	Immune
1	1-10	Upto 5	Resistant
2	11-25	6-10	Moderately resistant
3	26-50	11-20	Moderately susceptible
4	51-75	21-50	Susceptible
5	>75	>50	Highly susceptible

Per Disease Index (PDI) was calculated by using following formula proposed by Wheeler 1969.

$$PDI = \frac{\text{Sum of the individual disease ratings}}{\text{Number of fruits/ leaves observed} \times \text{Maximum disease grade}} \times 100$$

Table 1. Status of pomegranate diseases in Kachchh district of Gujarat during 2018-19.

District	Taluka	Village	Genotype	Bahar	Stage of the crop	Percent disease index Alternaria leaf and fruit spot	
						On leaf	On fruit
	Bhachau	Bhachau					
		local	Bhagwa sinduri	Mrig	Flowering & Fruiting	10.60	18.60
		Chobari	Bhagwa sinduri	Mrig	Flowering & Fruiting	16.60	36.50
		Gunatitpura	Bhagwa sinduri	Hasta	Flowering & Fruiting	6.50	11.80
		Kharoi	Bhagwa sinduri	Mrig	Flowering & Fruiting	9.80	21.50
		Manfara	Bhagwa sinduri	Mrig	Flowering & Fruiting	5.80	10.60
		May	Bhagwa sinduri	Mrig	Flowering & Fruiting	4.50	11.50
		RRS	Bhagwa sinduri	Hasta	Flowering & Fruiting	4.50	5.80
		Sangamner	Bhagwa sinduri	Mrig	Flowering & Fruiting	11.50	24.50
			Mean			8.72	17.60
Kuchchh	Anjar	Ambapar	Bhagwa sinduri	Mrig	Flowering & Fruiting	9.60	13.90
		Nagalpar	Bhagwa sinduri	Mrig	Flowering & Fruiting	8.90	14.50
		Navagam	Bhagwa sinduri	Mrig	Flowering & Fruiting	11.70	21.80
		Pashwadi	Bhagwa sinduri	Mrig	Flowering & Fruiting	5.60	12.90
		Satapar	Bhagwa sinduri	Mrig	Flowering & Fruiting	10.50	22.90
		Rampar	Bhagwa sinduri	Mrig	Flowering & Fruiting	5.90	13.45
			Mean			8.70	16.56
	Bhuj	Bharapar	Bhagwa sinduri	Mrig	Flowering & Fruiting	-	-
		Bhujodi	Bhagwa sinduri	Mrig	Flowering & Fruiting	-	-
		Chapreli	Bhagwa sinduri	Mrig	Flowering & Fruiting	5.61	6.58
		Dahinsara	Bhagwa sinduri	Mrig	Flowering & Fruiting	6.21	8.20
		Jawaharnagar	Bhagwa sinduri	Mrig	Flowering & Fruiting	6.50	9.81
			Mean			3.67	4.92
	Mundra	Dhrub	Bhagwa sinduri	Mrig	Flowering & Fruiting	4.50	7.80
		Gundala	Bhagwa sinduri	Mrig	Flowering & Fruiting	6.80	8.55
		Jarpara	Bhagwa sinduri	Mrig	Flowering & Fruiting	4.12	-
		Kundrodi	Bhagwa sinduri	Mrig	Flowering & Fruiting	-	-
		Pragpar	Bhagwa sinduri	Mrig	Flowering & Fruiting	6.72	8.80
			Mean			4.43	5.03
	Nakhat rana	Deshalpar	Bhagwa sinduri	Mrig	Flowering & Fruiting	3.50	6.50
		Gadani	Bhagwa sinduri	Mrig	Flowering & Fruiting	4.10	7.00
		Netra	Bhagwa sinduri	Mrig	Flowering & Fruiting	3.90	5.40
		Sanyra	Bhagwa sinduri	Mrig	Flowering & Fruiting	-	-
		Vithon	Bhagwa sinduri	Mrig	Flowering & Fruiting	4.60	7.80
			Mean			3.22	5.34

Isolation, identification, proving pathogenicity and studies on symptoms

Pathogen was isolated by standard tissue isolation technique and identified by microscopic observation by taking mycelial and spore character and standard descriptions (Ellis 1971). Pathogenicity was proved by Koch's postulates. Symptoms were correlated with the pathogen.

***In vitro* evaluation of different bioagents, botanicals and chemicals**

Different bioagents, botanicals and chemicals were screened against *A. alternata* of pomegranate.

Bioagents were screened by dual culture technique. Per cent inhibition of mycelial growth over control was calculated by using the formula of Vincent (1947) as follows :

$$I = \frac{C - T}{C} \times 100$$

Botanicals were screened by poison food technique as suggested by Nene and Thapliyal (1982).

Table 2. *In vitro* evaluation of bioagents against *A. alternate*. *Arcsine transformed values.

Antagonists	Inhibition of mycelial growth (%)
Bacillus subtilis	67.24 (55.09)*
Pseudomonas fluorescens	62.94 (52.50)
Trichodema harzianum	71.70 (57.87)
Trichoderma koningii	64.97 (53.11)
Trichoderma viride	83.92 (66.37)
SEm±	0.40
CD at 1%	1.20
CV%	1.39

Table 3. *In vitro* evaluation of botanicals against *A. alternate.* * Arcsine transformed values.

Botanical	Per cent inhibition of mycelial growth Concentration (%) 5	10	Mean
Allium sativum	5.93 (14.00)*	25.93 (30.60)	15.93 (22.30)
Azadirachta indica	36.30 (37.04)	58.52 (40.90)	47.41 (43.47)
Ocimum sanctum	11.85 (20.10)	26.67 (31.09)	19.26 (25.60)
Zingiber officinalis	63.33 (52.73)	63.70 (52.96)	63.52 (52.85)
Curcuma longa	23.33 (28.88)	63.33 (52.74)	43.33 (40.81)
Nimbecidine 1500 ppm	76.30 (60.87)	80.00 (63.44)	78.15 (62.16)
Lawsonia inermis L.	33.70 (35.48)	64.44 (53.40)	49.07 (44.44)
Murraya koenigii L.	42.22 (40.52)	66.67 (54.74)	54.44 (47.63)
Mean	36.62 (36.21)	56.16 (48.61)	
	B	C	B×C
SEm±	0.35	0.18	0.50
CD at 1%	1.02	0.51	1.44
CV%	2.51		

Cold aqueous extract of botanicals were prepared for screening. Fresh leaves or fruits or kernals of each test plant were collected and washed with tap water followed by distilled water. Then 100 g of fresh sample is crushed in a surface sterilized pestle and morter by adding 100 ml of sterile distilled water (1 : 1 w/v). The extracts was filtered through two layers of muslin cloth. Filtrate thus obtained was used as a stock solution and it was sterilized. To study the antifungal mechanism of plant extracts the poisoned food technique was five and ten ml of stock solution were mixed with 95 and 90 ml of sterilized molten PDA media respectively so as to get 5 and 10 percent concentration. The medium was thoroughly shaken. Twenty ml of medium was poured into each of the 90 mm sterilized Petriplates. Each plate were seeded with 5 mm mycelial discs taken from the periphery of eight day old *A. alternata* culture and were incubated at 28 ± 20°C till the growth of colony touches the periphery in control plate. The disc is placed upside down in the center of the Petriplates, so that the mycelium is in direct contact with the medium poisoned with the requisite plant extract at required concentration. Three replications were maintained for each treatment. Radial growth over the control was measured and percent inhibition of mycelial growth over control was calculated by using formula mentioned above.

Sixteen fungicides were tested in *in vitro* for their efficacy by following poison food technique against *A. alternata*. Fungicide suspension was prepared in PDA by adding required quantity of fungicides concentration. Twenty ml of poisoned medium was poured in each of the sterilized Petriplates. Mycelial disc of 5 mm was taken from the periphery of ten days old culture and placed in the center and incubated at 28±20°C till growth of the fungus touched the periphery in control plate. Suitable checks were maintained without the addition of any fungicide, three replications were maintained for each treatment. The colony diameter of the fungus was measured in two directions and average was worked out. The per cent inhibition of growth was calculated by using the formula mentioned above.

RESULTS AND DISCUSSION

Severity of the disease

Roving survey was conducted in the eight locations

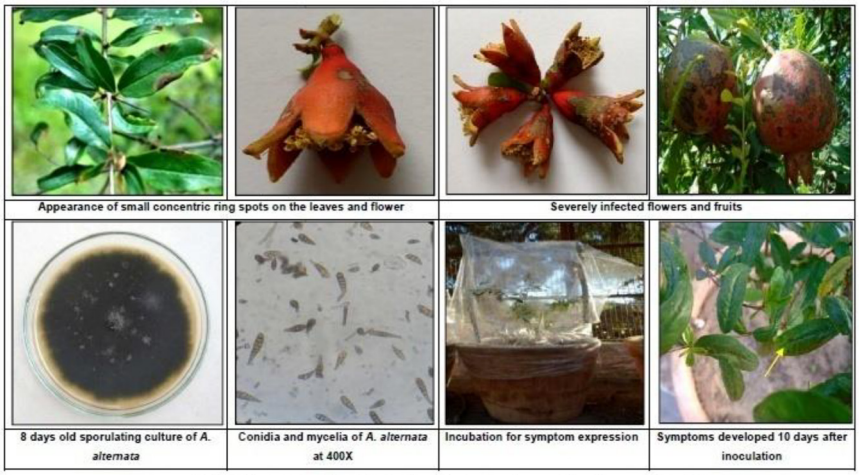

Plate 1. Studies on Alternaria leaf and fruit spot of pomegranate caused by *Alternatia alternata*.

of Bhacahu taluka, six locations of Anjar taluka, five locations of Bhuj taluka, five locations of Mundra taluka and five locations of Nakhatrana taluka during 2017-18, observed the alternaria leaf / fruit spot diseases of pomegranate. The disease severity was ranged from 0 to 16.60 % on leaf and 0 to 36.50 % on fruit. Maximum disease severity of alternaria leaf spot (16.60%) and fruit spot (36.5%) was observed in the Chobari village of Bhachau taluka, whereas Bharapar and Bhujodi (Bhuj taluka), Kundrodi (Mundra taluka) and Sanyra (Nakhatrana taluka) villages were found as disease free areas. The cultivar was Bhagwa sinduri in all the surveyed areas and pruning treatment was mrig bahar (Table 1). Infected leaves were acted as secondary source of inoculum the infection chain was continued from leaf to the fruit and further increase in severity is mighty be attributed to the presence of relative humidity more than 80% and temperature 25–35°C. These congenial environmental conditions for development of alternaria leaf spot/blight were also reported by Ahamad and Narain (2000) in bitter gourd and Murumkar *et al.* (2008) in safflower. Tetarwal *et al.* (2020) conducted survey in Banaskantha district of Gujarat and reported the maximum incidence of leaf spot (14.10%) and fruit spot (13.81%) in Bhabhar taluka of Banaskantha district.

Diseased samples showing the symptoms of small dark brown color spots with round or irregular concentric rings in the center were collected. Later these spots increases in the size, coalesced and affected leaves become yellow and dropped down. On the fruits they may cover larger fruit area but infection was restricted to rind of the fruit and arils were not infected. Symptoms observed during the survey have been presented in the photographs (Plate 1).

Samples collected during the survey were isolated. Based on the morphological characters pathogens was identified as *Alternaria alternata* (Fr.) Keissler and pathogenicity was proved on cultivar Bhagwa sinduri.

In vitro evaluation of different bio agents, botanicals and chemicals

In vitro evaluation study was conducted against *A. alternata,* significant mycelial inhibition was observed among different bio agents, botanicals and chemicals tested.

Five different bioagents were tested with four replications against *Alternaria alternata* under *in vitro* condition by dual culture technique. Among different bioagents tested, *Trichoderma viride* recorded the 83.92% inhibition, followed by *Trichoderma harzianum* (71.70%). Least inhibition of 62.94% was recorded by *Pseudomonas fluorescens* (Table 2). *Trichoderma* is hyperparasite it grows on the

Table 4. *In vitro* evaluation of contact fungicides against *A. alternate*. * Arcsine transformed values.

Fungicide	Per cent inhibition of mycelial growth Concentration (%) 0.1	0.2	0.3	Mean
Chlorothalonil 75% WP	53.70 (47.12)*	57.04 (49.05)	56.30 (48.62)	55.68 (48.26)
Mancozeb 75% WP	67.41 (55.19)	68.15 (55.64)	79.26 (62.91)	71.61 (57.91)
Propineb 70% WP	80.74 (63.97)	85.19 (67.36)	88.52 (70.19)	84.82 (67.17)
Copper oxychloride 50% WP	74.44 (59.63)	87.04 (68.90)	91.85 (73.41)	84.44 (67.31)
Mean	69.07 (56.48)	74.35 (60.24)	78.98 (63.78)	74.13 (60.17)
Fungicide	F	C	F×C	
SEm±	0.56	0.48	0.97	
CD at 1%	1.63	1.41	2.82	
CV	2.78			

pathogen by coiling around the hypha, by penetrating mycelium of the pathogen. It also produces the secondary metabolites like, dermin, trichodermin, viridin. These metabolites inhibited the pathogen by the antibiosis mechanism. Sanjeev *et al.* (2017), reported that all the strains of *Trichoderma harzianum* were inhibited the mycelial growth of *A. alternata,* causal agent of fruit rot of brinjal. Babu *et al.* (2000) reported that *Trichoderma harzianum* and *Trichoderma viride* were significantly superior in inhibiting the mycelial growth of *Alternaria solani*, causing leaf blight of tomato.

Eight different botanicals tested with three replications by poison food technique. Among different botanicals tested Nimbecidine 1500 ppm recorded the maximum inhibition (78.15%) and least (15.93%) was observed with the *Allium sativum*. In the interaction study, maximum inhibition of mycelial growth was observed with the Nimbecidine 1500 ppm that is 80.00% at 10% concentration and least inhibition (5.93%) was observed with the *Allium sativum* at 5% concentration (Table 3). Inhibitory action of Nimbecidine may be due to the presence of triterpenoid azadirchtin which retards the growth and activation of the pathogen. These results are in support with earlier work of Kotramma *et al.* (2017) reported that Nimbecidine is highly effective against the manage-

Table 5. *In vitro* evaluation of systemic fungicides against *A. alternate.* * Arcsine transformed values.

Fungicide	Per cent inhibition of mycelial growth Concentration (%) 0.05	0.1	0.15	Mean
Carbendazim 50% WP	60.37 (50.99)*	68.15 (55.64)	76.67 (61.12)	68.40 (55.91)
Hexaconazole 5% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Propiconazole 25% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Difenconazole 25% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Tebuconazole 250% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Azoxystrobin 25% SC	63.70 (52.95)	70.37 (57.02)	78.15 (62.13)	70.74 (57.37)
Pyraclostrobin 20% WG	66.67 (54.74)	70.00 (56.79)	78.52 (62.39)	71.73 (57.97)
Mean	84.39 (74.10)	86.93 (75.64)	90.48 (77.95)	
	F	C	F×C	
SEm±	0.22	0.14	0.38	
CD at 1%	0.62	0.41	1.08	
CV %	0.87			

Table 6. *In vitro* evaluation of combiproduct fungicides against *A. alternate.* * Arcsine transformed values.

Fungicide	Per cent inhibition of mycelial growth Concentration (%) 0.1	0.2	0.3	Mean
Carboxin 37.5% + Thiram 37.5 % WS	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Carbendazim 12% + Mancozeb 63% WP	80.37 (63.70)	85.93 (67.97)	85.93 (67.97)	84.07 (66.54)
Hexaconazole 4% + Zineb 68% WP	92.96 (74.62)	92.96 (74.62)	100.00 (90.00)	95.31 (79.74)
Captan 70% + Hexaconazole 5% WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Tebuconazole 50% + Trifloxystrobin 25% WG	92.59 (74.21)	100.00 (90.00)	100.00 (90.00)	97.53 (84.74)
Mean	93.19 (78.50)	95.78 (85.52)	97.19 (85.59)	
	F	C	F×C	
SEm±	0.37	0.29	0.65	
CD at 1%	1.08	0.84	1.87	
CV%	1.36			

ment of *Curvularia lunata* under *in vitro* condition.

Among the four contact fungicides tested Propineb 70% WP (84.82%) and *Copper oxychloride* 50% WP (84.44%) was found highly effective followed by Mancozeb 75% WP (71.61%). Increase in the concentration level, increased per cent inhibition of mycelial growth. Among three concentrations tested, 0.3% concentration showed maximum inhibition (78.98%). In F×C interaction study *Copper oxychloride* 50% WP at 0.3% concentration recorded maximum inhibition of mycelial growth (91.85%) and least (53.70%) was observed with the Chlorothalonil 75% WP at 0.1% concentration (Table 4). Vasudha *et al.* (2018), reported that *Copper oxychloride* was found as second best fungicide for inhibition of mycelial growth of *Alternaria alternata,* causing leaf and fruit spot of pomegranate.

Among the seven systemic fungicides tested, Hexaconazole 5% EC, Propiconazole 25% EC, Difenconazole 25% EC and Tebuconazole 250 % EC recorded cent per cent inhibition at all the concentrations tested and least inhibition of mycelial growth (68.40%) was observed with Carbendazim 50% WP. Increase in the concentration of fungicides, increased the per cent inhibition of mycelial growth, except in triazole fungicides where they are found cent per cent effective even at lower concentration. In the F×C interaction study also triazole recorded 100% inhibition of mycelial growth at 0.05 concentration (Table 5). Among five different combiproduct fungicides tested, 100% inhibition of mycelial growth was observed with Carboxin 37.5% + Thiram 37.5% WS and Captan 70% + Hexaconazole 5% WP at all the concentrations tested and in the interaction study least inhibition (80.37 %) was observed with the Carbendazim 12% + Mancozeb 63% WP at 0.1% concentration (Table 6). Among all the fungicides tested triazole fungicides (Hexaconazole, Propiconazol, Difenconazole and Tebuconazole) and Combiproducts containing the triazole fungicide as a one component are found best for the inhibition of the mycelial growth of the pathogen because these fungicides are very effective against the true fungi or the fungi synthesis ergosterol, they inhibit the demethylation process during the ergosterol synthesis hence they effectively inhibited the mycelial growth of the *A. alternata*. Carbendazim is effective against the hyaline spores of the fungi, Alternaria produces the colored spores hence Carbendazim is found least effective in inhibiting the mycelial growth of the fungi. Adesh *et al.* (2017) reported that the triazole fungicides were found best for inhibition of mycelial growth of *Alternaria alternata*

of pomegranate and Archana and Jamadar (2014) also reported that alternaria leaf spot of pomegranate can be effectively managed by using triazole fungicide (Propiconazole) as compared to strobilurin and other systemic fungicides. Vasudha *et al.* (2018), reported that Propiconazole, Hexaconazole, Penconazole and Difenconazole were recorded the maximum inhibition of mycelial growth of *A. alternata.*

CONCLUSION

The *Alternaria alternata* is one of the major pathogen contributing for fruit spots of pomegranate which reduces the market value. Severity of disease in entire district was ranged from 0-16.60% on leaf and 0-36.50% on fruit on cultivar Bhagwa sinduri. The bioagent *T. viride,* botanical Nimbecidine 1500 ppm and triazole fungicides effectively inhibited mycelial growth *A. alternata* in *in vitro* condition. These combination of bioagent, botanical and fungicides can be used as component of integrated management of alternaria leaf and fruit spot in field condition also.

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