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New novel fungicides for management of anthracnose of pomegranate caused by *Colletotrichum gloeosporioides*

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Abstract
Management of pomegranate anthracnose has been critical and essential part of cultivation in every pomegranate orchard. Often this fungal disease management was done using fungicides as no resistant cultivars are available. But frequent sprays of fungicides have posed threats of high fungicide resides. Hence, the present investigation was undertaken to evaluate the new and novel fungicide (combi product) molecule with two different modes of action along with already recommended single mode of action fungicides. The experiment was done for two years and found that foliar spray of Aoxystrobin 8.3%+Mancozeb 66.7 percent at 3.5 gm/l was most effective with lowest mean Percent Disease Index (PDI) of 4.90 percent. This treatment also had the highest disease control (88.5%) compared to other treatments. Same fungicide at 3 g/l was also found effective and on par with 3.5 g/l dosage. This fungicide is of dual mode of action and has an advantage of delaying the development of resistance by the pathogen towards the fungicide. Due to its dual mode of action the spray frequencies will also get reduced due to prolonged effect of the fungicide. Foliar spry of Azoxystrobin 23 percent SC was also on par with the Aoxystrobin 8.3%+Mancozeb 66.7 percent but the cost benefit analysis showed that spraying of Aoxystrobin 8.3%+Mancozeb 66.7 percent would be most economical with maximum returns with an additional advantage of prolonged effect and avoiding the fungicide residues in the fruits with reduced number of sprays.

Keywords: Pomegranate, anthracnose, colletotrichum, management and fungicides

Introduction
Pomegranate is most cultivated sub-tropical fruit crop across India and other countries. In India, Karnataka, Maharashtra, Rajasthan, Madhya Pradesh, and Telangana are dominating in area and production of pomegranate. The fruit is known for its multiple health benefits. The small pink seeds inside pomegranate fruit are called arils and are the edible portion of the fruit. Pomegranate seeds contain two major compounds responsible for their health benefits – punicalagin and punicic acid. Punicalagins are a group of antioxidants commonly found in pomegranate juice and peel, whereas punicic acid, found in seed oil is the main fatty acid. "Pomegranates are high in fiber and anthocyanins, which are strong antioxidants and responsible for their beautiful ruby red color. Pomegranate juice is beneficial for people with type 2 diabetes, inflammatory conditions, and other health issues. It may also boost digestion, memory and help prevent cancer (Puneeth, 2020) [7]. One pomegranate (282 g) provides 234 calories, 4.7 g of protein, 52.7 g of carbohydrates and 3.3 g of fat. Pomegranate seeds, or arils are a very good source of fibre and rich in potassium, phosphorus, magnesium, and calcium. With many more health advantages being identified each day, the demand for pomegranate fruits is quite high. A vast value chain has developed in pomegranate cultivation to consumer delivery. However, the latter is driven by man and machines but cultivation is very much driven and dependant by on biological factors. Among the biotic factors, diseases are hindering the commercial cultivation of pomegranate. These diseases account for more than 40 percent of total cost of cultivation per unit area of pomegranate.
Major diseases that affect pomegranate are bacterial blight (*Xanthomonas axonopodis pv punicae*), anthracnose (*Colletotrichum gloeosporoides*) and wilt complex (*Ceratocystis fimbriata*) affects root in association with shot hole borer (*Xyleborus perforans* (Wollastan)) and other fungal pathogens (Munhuweyi *et al*. 2016) [5]. Bacterial blight and wilt together are responsible for 60 percent decline in pomegranate yield. In addition to this, the quality of fruit

is affected by anthracnose disease. This disease is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. is one of the most serious diseases of pomegranate affects both quality and marketability of fruits. In India, Chandra and Tondon (1965) [1] first time noticed the *C. gloeosporioides* causing pomegranate leaf spot and fruit rot.

The *Colletotrichum* infects pomegranate leaves and fruit, causing anthracnose leaf blight and fruit rot, respectively. Fruits infected by this pathogen become unmarketable, leading to economic losses (Sataraddi *et al*. 2011; Sharma *et al*. 2015, Munhuweyi *et al*., 2016) [8, 9, 5]. This disease is favoured by high humidity, ranging from 62 to 95 percent and temperatures ranging from 20 to 30 °C, which are common in tropical and subtropical regions. Pomegranate anthracnose symptoms start as small circular leaf spots with yellow halos. Infected leaves then become chlorotic and drop from the tree, leading to premature defoliation. Fruit symptoms are characterized by brown lesions that later progress through the rind and arils, resulting in fruit decay. Gray to orange spore masses often become visible on lesions of either green or ripe fruit (Thomidis, 2014; Munhuweyi *et al*. 2016) [10, 5].

Management of pomegranate anthracnose is top priority of every grower till the fruits are harvested. Since, the pathogen causes heavy yield losses especially the quality of fruits. Many fungicides are being employed for management of the disease but effective control has remained still a challenge. The fungicides also require frequent sprays. Pomegranate being edible fruit of niche market, the present investigation was undertaken to know the efficacy of new and novel fungicide molecules in combined form having two different mode of actions against anthracnose disease in comparison with other single mode of action fungicide molecules already being used.

Materials and Methods

A field experiment was conducted for two years to assess the efficacy of new fungicide molecule (Aoxystrobin 8.3%+Mancozeb 66.7%) 75WP along with other fungicides against the anthracnose disease of pomegranate in comparison with control in a pomegranate orchard of Kesar cultivar. Six different fungicides in eight treatments and a control plot were maintained. The fungicides were sprayed during Ambebahar season.

Treatments	Treatment details	Dosage
T_1	Aoxystrobin 8.3%+Mancozeb 66.7%	2.5 g/l
T_2	Aoxystrobin 8.3%+Mancozeb 66.7%	3 g/l
T_3	Aoxystrobin 8.3%+Mancozeb 66.7%	3.5 g/l
T_4	Azoxystrobin 23% SC	1 ml/l
T_5	Mancozeb 75% WP	5 g/l
T_6	Hexaconazole 5Ec	1 ml/l
T_7	Tebuconazole 25.9EC	1 ml/l
T_8	Carbendazim 50WP	1 g/l
T_9	Untreated check	

Nine treatments were imposed including an untreated control. Total three fungicide sprays were given. First spray was given immediately after the onset of anthracnose disease. Subsequently two more spray were given at an interval of 15 days. Each treatment had three replications. The Percent Disease Index (PDI) and percent disease reduction over control were calculated and arcsine transformed data were analysed statistically to derive the conclusion. The benefit to cost ratio was calculated to derive the conclusion.

Results and Discussion

Different fungicides sprayed against anthracnose disease in pomegranate varied in their action against the disease. Among them, a new and novel fungicide molecule consisting two fungicide molecules azoxystrobin and mancozeb with different modes of action (Aoxystrobin 8.3%+Mancozeb 66.7%) tested at three different concentrations. Two concentrations of this fungicide 3.0 g/l and 3.5 g/l showed very promising response against the anthracnose disease for two years of evaluation. During both the years, treatment T_3 involving Aoxystrobin 8.3%+Mancozeb 66.7% at 3.5 gm/l recorded lowest PDI of 3.75 and 5.80 percent in first and second year while reducing the disease incidence by 90.35 and 88.12 percent over control respectively. Mean disease incidence by this treatment was 4.90 percent. Whereas in control 38.85 and 48.83 PDI was observed in first and second years with an average PDI of 42.62 percent. The next best treatment was T_2 involving foliar spray of same fungicide Aoxystrobin 8.3%+Mancozeb 66.7% at 3.0 gm/l recorded on par PDI in both the years as that of T_3. Further, foliar spry of Azoxystrobin 23% SC (T_4) was also found next promising fungicide which reduced the disease incidence by 85.05 and 87.14 percent during first and second year respectively with least PDI of 5.81 and 6.28 compared to control. With the second lowest disease incidences, the treatment T_2 recorded the highest and marketable quality fruit yield of 16429kg/ha and 17917kg/ha during first and second year. The second highest fruit yield was recorded in T_4 15853kg/ha and 17738 kg/ha in first and second years respectively. In control the lowest fruit yield of 5232kg/ha and 5760kg/ha was noticed in first and second year respectively. The results are conclusive with maximum B:C ratio of 11.59 in T_2 compared to 4.02 in untreated control. The other treatments T_4 and T_3 had on par B:C ratio (11.49 & 10.95).

The experiment revealed the potential benefits of new and novel fungicide molecule with two modes of actions contact and systemic by Mancozeb and Aoxystrobin respectively together in single product in wettable powder formulations very ideal for management of Anthracnose of pomegranate. The Azoxystrobin in this fungicide is known to inhibit the mitochondrial respiration in pathogen by blocking the electron transport chain in the inner mitochondrial membrane (Kanako Inoue *et al*., 2012) [4]. The inhibition of respiratory electron transfer chain causes loss of ATP synthesis, subsequently preventing ATP-consuming metabolic activity. On the other hand Mancozeb reacts with, and inactivates, the sulfhydryl groups of amino acids and enzymes within fungal cells, resulting in disruption of lipid metabolism, respiration, and production of adenosine triphosphate (Tomlin, 2003) [11]. These multiple modes of actions proved very promising in reducing the pathogen growth and disease incidence. In other studies, aimed at pomegranate anthracnose disease management using fungicides, combined fungicides with two different modes of action were proved superior over single molecule application both *in vitro* and *in vivo* (Ekabote, and Narayanswamy, 2019; Vandana, *et al*., 2021) [2, 12]. To get the superior quality pomegranate fruits, the new fungicide molecule Mancozeb and Aoxystrobin was found very promising and these kind of new and multiple modes of action molecules are also required to overcome the risk of development of resistance in the pathogen due to continued use of fungicides with single mode of action or site of action more frequently. Sometimes, the pathogen can adapt to

fungicide treatments by mutations leading to resistance development and loss of fungicide efficacy (Hahn, 2014). There is also a risk of accumulating high fungicide residue due to frequent and unscientific sprays (Grewal *et al*., 2017; Nikhat Khan *et al.,* 2020) [13, 6]. To overcome these bottlenecks and reduce the spray frequencies with low or no residue levels, new and novel fungicide (Aoxystrobin 8.3%+Mancozeb 66.7%) identified in the present study shall be adopted for successful control of anthracnose in pomegranate at field level.

Table 1: Effect of new combination fungicide molecule in comparison with other fungicides against anthracnose disease of pomegranate (2019-20)

Treatment Nos	Treatment details	Dosage	PDI (%)	Disease reduction over control	Fruit yield (kg/plant)	Fruit Yield (kg/ha)
T_1	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	2.5 g/l	11.85 (20.13)	69.50	12.42	9933
T_2	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	3.0 g/l	5.60 (13.67)	85.59	20.54	16429
T_3	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	3.5 g/l	3.75 (11.16)	90.35	19.51	15610
T_4	Azoxystrobin 23% SC	1.0 ml/l	5.81 (13.95)	85.05	19.82	15853
T_5	Mancozeb 75% WP	5.0 g/l	16.70 (24.11)	57.01	14.09	11274
T_6	Hexaconazole 5Ec	1.0 ml/l	10.56 (18.95)	72.82	19.56	15644
T_7	Tebuconazole 25.9EC	1.0 ml/l	9.61 (18.06)	75.26	15.33	12264
T_8	Carbendazim 50WP	1.0 g/l	12.85 (20.99)	66.92	13.54	10828
T_9	Untreated check		38.85 (38.54)		6.54	5232
	S.Em+		1.03		1.06	
	C.D.@ 5%		3.19		3.26	
	CV		13.91		11.82	

Table 2: Effect of new combination fungicide molecule in comparison with other fungicides against anthracnose disease of pomegranate (2020-21)

Treatment Nos	Treatment details	Dosage	PDI (%)	Disease reduction over control	Fruit yield (kg/plant)	Fruit Yield (kg/ha)
T_1	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	2.5 g/l	14.42 (22.61)	70.47	13.83	11064
T_2	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	3.0 g/l	6.64 (14.99)	86.40	22.40	17917
T_3	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	3.5 g/l	5.80 (13.91)	88.12	21.47	17176
T_4	Azoxystrobin 23% SC	1.0 ml/l	6.28 (14.49)	87.14	22.17	17738
T_5	Mancozeb 75% WP	5.0 g/l	21.27 (27.69)	56.44	14.83	11861
T_6	Hexaconazole 5Ec	1.0 ml/l	16.45 (24.22)	66.31	19.41	15528
T_7	Tebuconazole 25.9EC	1.0 ml/l	11.38 (19.67)	76.69	17.28	13821
T_8	Carbendazim 50WP	1.0 g/l	16.94 (24.06)	65.31	14.13	11304
T_9	Untreated check		48.83 (44.27)		7.20	5760
	S.Em+		1.48		1.22	
	C.D.@ 5%		4.53		3.77	
	CV		15.65		12.50	

Table 3: Effect of new combination fungicide molecule in comparison with other fungicides against anthracnose disease of pomegranate (Pooled two years)

Treatment Nos	Treatment details	Dosage	PDI (%)	Disease reduction over control	Fruit yield (kg/plant)	Fruit Yield (kg/ha)
T_1	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	2.5 g/l	13.44 (21.49)	68.47	13.12	10499
T_2	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	3.0 g/l	5.84 (14.36)	86.30	21.47	17173
T_3	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	3.5 g/l	4.90 (12.78)	88.50	20.49	16393
T_4	Azoxystrobin 23% SC	1.0 ml/l	5.94 (14.09)	86.06	21.00	16796
T_5	Mancozeb 75% WP	5.0 g/l	19.47 (26.17)	54.32	14.46	11568
T_6	Hexaconazole 5Ec	1.0 ml/l	13.61 (21;64)	68.07	18.78	15027
T_7	Tebuconazole 25.9EC	1.0 ml/l	10.51 (18.91)	75.34	16.30	13043
T_8	Carbendazim 50WP	1.0 g/l	15.12 (22.87)	64.52	14.15	11316
T_9	Untreated check		42.62 (40.74)		6.87	5496
	S.Em+		0.95		0.95	
	C.D.@ 5%		2.91		2.92	
	CV		11.21		10.08	

Table 4: Assessment of cost benefit ratio in management of pomegranate anthracnose disease by different fungicides.

Treatment Nos	Treatment details	Dosage	Cost of cultivation (Rs./ha)	Fruit yield (kg/ha)	Gross returns (Rs./ha)	Net returns (Rs./ha)	BC ratio
T_1	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	2.5 g/l	88000	10499	524950	436950	5.97
T_2	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	3.0 g/l	88900	17173	1030380	941480	11.59
T_3	Aoxystrobin 8.3%+Mancozeb 66.7% (Avancer glow)	3.5 g/l	89800	16393	983580	893780	10.95
T_4	Azoxystrobin 23% SC	1.0 ml/l	87700	16796	1007760	920060	11.49
T_5	Mancozeb 75% WP	5.0 g/l	82750	11568	694080	611330	8.39
T_6	Hexaconazole 5 EC	1.0 ml/l	82600	15027	901620	819020	10.92
T_7	Tebuconazole 25.9 EC	1.0 ml/l	83800	13043	782580	698780	9.34
T_8	Carbendazim 50 WP	1.0 g/l	82600	11316	678960	596360	8.22
T_9	Untreated check	-	82000	5496	329760	247760	4.02

Conclusion

The new and novel fungicides is very effective in reducing the anthracnose disease of pomegranate and shall help in reducing the number of sprays required with its dual mode of action.

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