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Screening and Field Validation of Fungicides for Control of Alternaria Spot in Pomegranate (*Punica granatum* L.)

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Abstract

Alternaria leaf and fruit spot of pomegranate caused by *Alternaria alternata* is a major disease affecting yield and quality. The pathogen was isolated and identified based on cultural, morphological, and pathogenicity characteristics. *In vitro* evaluation of fungicides showed that azoxystrobin (0.1%) was most effective, recording maximum mycelial inhibition (90.11%), followed by trifloxystrobin + tebuconazole (88.78%). Field studies also confirmed that azoxystrobin (0.1%) significantly reduced disease intensity (5.07%) and recorded higher yield (177.85 q ha⁻¹). These results indicate that effective management of Alternaria spot can be achieved through the use of suitable fungicides, particularly azoxystrobin.

Keywords: Pomegranate, azoxystrobin, tebuconazole, *Alternaria*

1. Introduction

Pomegranate (*Punica granatum* L.) is an important fruit crop cultivated in arid and semi-arid regions due to its adaptability to adverse environmental conditions (Jalilop, 2010; Levin, 2006) [8, 10]. In India, it has gained significant commercial importance, with Maharashtra being the leading producer (Anonymous, 2023) [2]. The fruit is valued for its nutritional and medicinal properties, as it is rich in antioxidants, vitamins, and bioactive compounds (Viuda-Martos *et al.*, 2010) [13].

However, pomegranate production is severely affected by various diseases, among which Alternaria leaf and fruit spot caused by *Alternaria alternata* has emerged as a major threat (Gade and Ghewande, 2010) [7]. The disease leads to significant yield losses and deterioration of fruit quality, especially under warm and humid conditions (Archana and Jamadar, 2014) [3]. Symptoms include dark, concentric lesions on leaves and fruits, resulting in defoliation, fruit rot, and reduced market value (Tziros *et al.*, 2008) [12].

Although fungicides are widely used for disease control, concerns regarding resistance development, environmental safety, and cost have increased (Adesh Kumar *et al.*, 2017) [1]. Therefore, a comprehensive study on the pathogen, its epidemiology, and effective management strategies is essential. The present investigation was undertaken to address these aspects and to develop sustainable disease management practices for pomegranate.

2. Isolation and Purification of Pathogen (*Alternaria alternata*).

The pathogen was isolated from naturally infected pomegranate leaves and fruits showing typical symptoms of Alternaria spot (Plate 3,4). The infected plant parts were first washed thoroughly under running tap water to remove surface contaminants and then air-dried. Small tissue bits (2–5 mm) were cut from the junction of healthy and diseased portions using a sterile scalpel. The excised tissue pieces were surface sterilized with 0.1 per cent mercuric chloride solution for one minute, followed by three successive washings with sterile distilled water to remove traces of the sterilant. The sterilized tissues were then blot-dried on sterile filter paper and aseptically transferred onto potato dextrose agar (PDA) medium in Petri plates.

The inoculated plates were incubated at 27 ± 2 °C in an inverted position. Fungal growth was observed within 24 hours of incubation, initially appearing white and later turning grey to

black. The emerging colonies were sub-cultured on fresh PDA medium to obtain pure cultures of the pathogen, which were maintained on PDA slants for further studies.

3. Morphology of *Alternaria alternata*

The morphological characteristics of the pathogen were studied on potato dextrose agar (PDA) medium. The colony initially appeared whitish, which later turned grey to black due to profuse sporulation. The growth was cottony to velvety in texture with irregular margins, and the reverse side of the colony exhibited dark pigmentation (Plate 1). Microscopic examination revealed that the mycelium was septate, branched, and light to dark brown in color. Conidiophores were short, septate, and borne singly or in small groups, measuring approximately 20–60 µm in length and 3–6 µm in width. They were pale to dark brown and slightly swollen at the tip. The conidia were dark brown, produced in chains, and exhibited characteristic obclavate to ellipsoidal shape. They measured about 20–60 µm in length and 7–18 µm in width, with a beak length of 2–10 µm. The conidia were multicellular with 3–10 transverse and 1–5 longitudinal septa (muriform) and possessed a distinct beak (Plate 2). These morphological features confirmed the pathogen as *Alternaria alternata*.

4. Pathogenicity Test

Pathogenicity of the isolated fungus was confirmed by inoculating a spore suspension of *Alternaria alternata* on healthy pomegranate leaves/fruits under humid conditions,

while control samples were maintained with sterile water. Typical symptoms of *Alternaria* spot, such as dark necrotic lesions with concentric rings, developed on inoculated samples (Plate 5), whereas no symptoms appeared in control (Plate 6). The pathogen was successfully re-isolated and found identical to the original culture, thus fulfilling Koch's postulates.

5. Efficacy of Fungicides against *Alternaria alternata* under *In vitro* Conditions

The *in vitro* evaluation of fungicides against *Alternaria alternata* revealed significant differences in their efficacy, with mycelial growth inhibition ranging from 66.44 to 90.11 per cent.

Azoxystrobin 23% SC (0.1%) recorded the highest inhibition (90.11%) with minimum mycelial growth (8.9 mm), and was statistically at par with tebuconazole 50% + trifloxystrobin 25% (0.05%) showing 88.78 per cent inhibition (10.1 mm). This was followed by meritam + pyraclostrobin (86.33%), metalaxyl-M + mancozeb (81.11%), difenoconazole (74.67%), Kasugamycin + copper oxychloride (70.89%), and propineb (68.56%). The lowest inhibition was recorded with Kitazin (66.44%) with maximum mycelial growth (30.2 mm) (plate 7) (Table 1). These findings are in agreement with earlier reports by Dambrauskiene (2006) [4], Kaur *et al.* (2020) [9], and Adesh Kumar *et al.* (2017) [11], who also reported high efficacy of azoxystrobin and its combinations against *Alternaria* spp. under *in vitro* conditions.

Table 1: *In vitro* evaluation of fungicides against *Alternaria alternata*

Tr. No.	Treatment Details	Mean growth (mm)	% Inhibition
T ₁	Azoxystrobin 23% SC @ 0.1%	8.9	90.11
T ₂	Difenconazole 25% EC @ 0.1%	22.8	74.67
T ₃	Kitazin 48% EC @ 0.2%	30.2	66.44
T ₄	Propineb 70% WP @ 0.3%	28.3	68.56
T ₅	Kasugamycin 5% + Copper oxychloride 45% WP @ 0.1%	26.2	70.89
T ₆	Metalaxyl M 4% + Mancozeb 64% WP @ 0.25%	17.0	81.11
T ₇	Meritam 55% + Pyraclostrobin 5% WG @ 0.15%	12.3	86.33
T ₈	Trifloxystrobin 25% + Tebuconazole 50% @ 0.05%	10.1	88.78
T ₉	Control	90.0	00.00
	SE(m ±)	1.10	
	CD at 1%	3.31	

6. *In vivo* evaluation of fungicides against *Alternaria alternata*

All fungicidal treatments significantly reduced *Alternaria* leaf and fruit spot intensity compared to the untreated control. Initial disease intensity ranged from 2.13 to 3.20 per cent before spraying.

After the first spray, disease intensity ranged from 2.93 to 19.20 per cent. Azoxystrobin (0.1%) recorded the lowest intensity (2.93%), followed by trifloxystrobin + tebuconazole (3.20%) and meritam + pyraclostrobin (4.27%), whereas propineb was least effective (8.27%).

Following the second spray, disease intensity ranged from 4.27 to 28.80 per cent. Azoxystrobin (4.27%) remained most effective, followed by trifloxystrobin + tebuconazole

(4.80%) and meritam + pyraclostrobin (5.87%), while propineb showed lowest efficacy (12.27%).

After the third spray, disease intensity varied from 5.07 to 37.87 per cent. Azoxystrobin (5.07%) consistently recorded the lowest disease intensity, followed by trifloxystrobin + tebuconazole (5.60%) and meritam + pyraclostrobin (9.07%). The untreated control showed maximum disease intensity (37.87%), while propineb remained least effective (13.87%).

These results (Table 2) are in agreement with earlier findings reported by Pasche *et al.* (2004) [11], Wang *et al.* (2016) [14], Fagodiya *et al.* (2022) [6], and Chakrabarty *et al.* (2025) [4], who also observed superior efficacy of azoxystrobin and its combinations against *Alternaria* spp.

Table 2: *In vivo* evaluation of fungicides against *Alternaria alternata*

Tr. No.	Treatments	Disease Intensity*						
		Initial	15 DAFS		15 DASS		15 DATS	
			PDI	PDC	PDI	PDC	PDI	PDC
T ₁	Azoxystrobin 23% SC @ 0.1%	2.40 (8.34)*	2.93 (9.69)	84.7	4.27 (11.83)	85.2	5.07 (12.94)	86.6
T ₂	Difenconazole 25% EC @ 0.1%	2.67 (8.89)	6.67 (14.84)	65.2	8.53 (16.88)	70.4	10.13 (18.48)	73.2
T ₃	Kitazin 48% EC @ 0.2%	2.13 (7.97)	7.73 (16.06)	59.7	10.13 (18.47)	64.8	11.73 (19.96)	69.0
T ₄	Propineb 70% WP @ 0.3%	2.93 (9.36)	8.27 (16.64)	56.9	12.27 (20.46)	57.4	13.87 (21.83)	63.3
T ₅	Kasugamycin 5% + Copper oxychloride 45% WP @ 0.1%	3.20 (8.40)	7.20 (15.41)	62.5	8.80 (17.14)	69.4	10.40 (18.72)	72.5
T ₆	Metalaxyl M 4%+ Mancozeb 64% WP @ 0.25%	2.40 (8.52)	5.87 (13.88)	69.4	8.81 (17.20)	69.4	9.87 (18.24)	73.9
T ₇	Meritam 55% + Pyraclostrobin 5% WG @ 0.15%	2.93 (8.24)	4.27 (11.45)	77.7	5.87 (13.74)	79.6	9.07 (17.41)	76.0
T ₈	Trifloxystrobin 25% + Tebuconazole 50% @ 0.05%	2.67 (8.89)	3.20 (9.95)	83.3	4.80 (12.46)	83.3	5.60 (13.53)	85.2
T ₉	Control	3.20 (9.77)	19.20 (25.86)	-	28.80 (32.28)	-	37.87 (37.90)	-
	S.E.(m) ±	2.33	1.36		1.4		1.19	
	C.D. at 5%	NS	4.14		4.23		3.62	

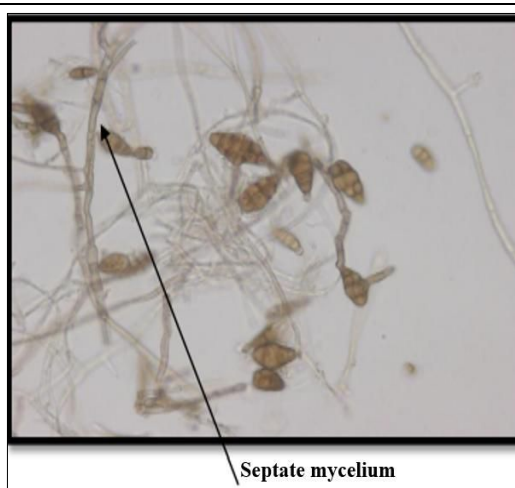
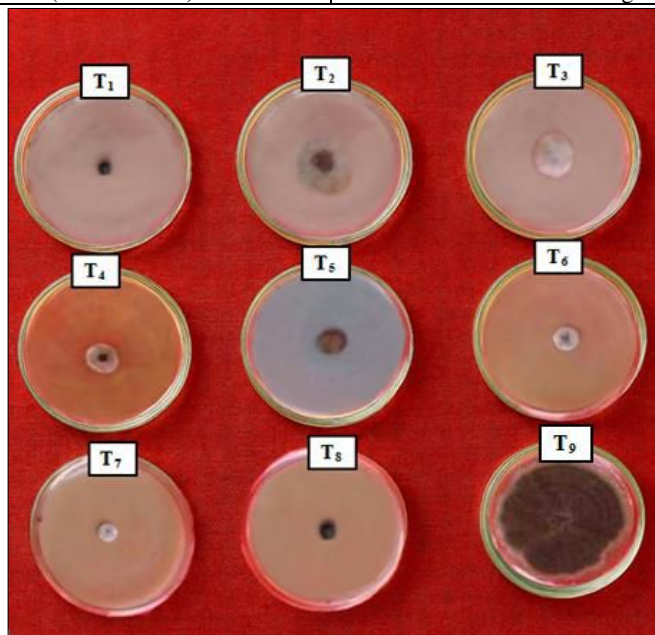
**Plate 1:** Pure culture of *A. alternata***Plate 2:** Microphotograph of *A. alternata***Plate 3:** Infected Pomegranate Fruits showing *Alternaria* spot symptoms.**Plate 4:** Infected Pomegranate Leaves showing *Alternaria* spot symptoms.



Plate 5: Pathogenicity test (Uninoculated)



Plate 6: Pathogenicity test (Inoculated)

Plate 7: *In vitro* evaluation of Fungicides against *Alternaria alternata*

7. Conclusion

The present investigation confirmed *Alternaria alternata* as the causal agent of leaf and fruit spot of pomegranate based on cultural, morphological, and pathogenicity studies. The pathogen produced characteristic symptoms upon artificial inoculation, thereby fulfilling Koch's postulates.

In vitro evaluation of fungicides revealed that azoxystrobin 23% SC (0.1%) was the most effective in inhibiting mycelial growth, followed by trifloxystrobin + tebuconazole and meritam + pyraclostrobin. Field studies further substantiated these findings, wherein azoxystrobin recorded the lowest disease intensity and highest fruit yield, and was statistically at par with trifloxystrobin + tebuconazole.

Overall, the study demonstrates that effective management of *Alternaria* spot of pomegranate can be achieved through the application of azoxystrobin or its combination with triazole fungicides. Adoption of these fungicidal treatments can significantly reduce disease severity and enhance yield under field conditions.

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