**Screening of newer generation fungicides against *Alternaria solani* causing early blight in tomato under *in vitro* condition**

**ABSTRACT**

Tomato (*Solanum lycopersicum* L.) is a globally vital horticultural crop, yet its productivity is severely threatened by early blight, caused by *Alternaria solani*. In a 2024 field survey across districts in Tamil Nadu, disease incidence ranged from 25.4% to 53.9% Per cent disease index, with the pathogen affects 60% in tomato field. *Alternaria solani* isolates were characterized morphologically, confirming typical septate, branched mycelia and muriform conidia. *In vitro* antifungal screening of eight newer-generation fungicides revealed that achieved the highest inhibition zone (48.15%), followed by difenoconazole at 42.73%, while hexaconazole exhibited the lowest activity (21.78%). These findings align with global studies demonstrating strobilurins’ superior efficacy in managing *A. solani*. The results suggest that azoxystrobin, either alone or in combination with triazoles, offers a promising tool for integrated early blight control in tomato cultivation. However, field trials, optimum dosing regimens, resistance monitoring, and integration with cultural practices are recommended to develop sustainable disease management strategies in tropical and subtropical regions.

**Key words:** Tomato, *Alternaria solani*, Newer generation fungicides**,** Antagonist

**INTRODUCTION**

Tomato (*Solanum lycopersicum L.*) is one of the most popular vegetable crops grown in the world, next to potato. It is a versatile ingredient commonly enjoyed as a fresh vegetable. It can be processed and canned in various forms, including paste, juice, sauce and powder, or preserved whole (barone and frusciante, 2007). “The ripe fruits are good source of vitamin A, B and C which add wide varieties of colour and flavour to the food. Recently, it has more active ingredients to recover the natural matrix for pharmaceutical and [nutraceutical](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/nutraceutical) applications” ([Madia *et al*., 2021](https://www.sciencedirect.com/science/article/pii/S0308814623024810#b0080)). In India, the maximum annual production and productivity of 2,12,38,000 and 24.33 metric tonnes (ministry of agriculture &; farmers welfare, Govt. of India, 2023). “India ranked as the fourth country in the world in tomato production after China, United States and Turkey. Tomato is one of the most widely cultivated [fruit crops](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fruit-crops), with a world annual production of over 187 million metric tons in 2021” (Matei *et al.,* 2021).

“Tomato crops opposed significant challenges from both abiotic stresses, such as extreme temperatures, salinity, drought, moisture fluctuations and environmental pollution, and biotic stresses, including various pests and diseases from seedling emergence to harvest. Numerous diseases impact tomatoes, particularly those caused by fungi, bacteria, viruses, and nematodes” (Mark and Brooke, 2006; Abada *et al*., 2008). Among these, “fungal diseases like Alternaria blight (*Alternaria sp*.), late blight *(Phytophthora infestans*), Septoria leaf blight (*Septoria lycopersici*), powdery mildew *(Oidiopsis taurica)*, Fusarium wilt (*Fusarium* *oxysporum* f. sp. *lycopersici*), collar rot *(Sclerotium rolfsii*) and damping-off *(Pythium sp.)* pose significant threats to production and lead to considerable economic losses. In Algeria, *Alternaria* blight caused by *Alternaria alternata* results in disease intensities of 46% to 90%, severely affecting fruit yield” (Bessadat *et al.,* 2014).

The genus *Alternaria* includes soil-borne pathogens, with several species like *Alternaria solani* identified as significant plant pathogens. First documented on potato plants by Jones and Grout in 1882, *A. solani* belongs to the phylum Ascomycota, class Othideomycetes, order Pleosporales, and family Pleosporaceae (Alhussaen, 2012). It typically infects solanaceous crops, including potatoes, tomatoes, eggplants, and peppers (Carneiro *et al.,* 2010). *A. solani* is the causal agent of early blight in tomatoes (Madden *et al.,* 1978), making these plants particularly vulnerable to infection (Chaerani *et al.,* 2007). Early blight is one of the most common and damaging fungal diseases, leading to premature leaf drop and significant losses in fruit quality and quantity (Holm *et al.,* 2003). It is especially problematic in warm, humid tropical and subtropical regions with factors like crowded planting, heavy rainfall, and prolonged leaf wetness contributing to disease development (Gondal *et al.,* 2012). Ultimately, *Alternaria* blight reduces the photosynthetic area of plants, further impacting their growth and yield (Madden *et al.,* 1978).

Managing *Alternaria* blight is challenging due to the pathogen's broad host range, variability in pathogenicity, and extended active phases in its disease cycle. The organism is soil-borne and disperses through the air, leading to consequence like *Alternaria* blight, collar rot, and fruit rot in tomatoes (Datar and Mayee, 1981). Recent fungicides tend to be highly specific and function through a single mode of action. As a result, it is essential to create new, effective fungicides that employ innovative modes of action to improve both the quality and quantity of tomato production (Sahu *et al.,* 2013; [Rex *et al*., 2019](https://www.sciencedirect.com/science/article/pii/S0308814623024810#b0100)).

**MATERIALS AND METHODS**

**Survey and Disease Incidence**

Survey was conducted in different tomato-growing areas of Tamil Nadu, India during the year 2024 and the disease incidences were recorded. The survey includes the observation of symptoms over the time and the Per cent Disease Index was calculated. The disease severity was assessed per plot by using 0-9 scales. Alternaria leaf spot infected leaf samples were collected from different tomato growing areas of Tamil Nadu for isolation of the fungus, *Alterneria solani.*

Percent disease index (PDI) was calculated as per the following formula given by Wheeler (1969).

PDI = Sum of individual ratings/ Number of leaves observed \* 100/ Maximum grade

**Isolation of *Alterneria solani***

The diseased leaf was first washed with tap water to remove dust and other contaminants. The infected portion was cut into small bits and surface sterilized with 10 per cent sodium hypochlorite for 5-10 minutes. In order to remove the residue of the chemical, the tissue bits were washed with three times of sterile distilled water. The surface sterilized bits were placed on Potato Dextrose Agar (PDA) medium in sterilized Petri dishes. These plates were incubated at room temperature (28 ± 2°C) for seven days. After incubation, the cultures were purified by hyphal tip method and the fungal cultures were maintained separately in sterile Perti plates.

**Morphological Characterization of *Alterneria solani***

The morphological characterization of all the isolates of *Alterneria solani*, was isolated from the spotted leaves using PDA medium and sub cultured using the single hyphal tip method and incubated at 28°C for 7 days. Initially, mycelium are septate, branched, hyaline and its darken in colour. They produce branched septatemycelium and conidiophores produce muriform conidia it may singly or in a chain (Naik *et al.,* 2010).

***In vitro* screening of newer generation fungicide against *Alterneria solani* (Dual culture technique)**

The antagonistic activity of newer generation fungicide against *Alterneria solani* was evaluated using the standard dual culture technique (Dennis and Webster, 1971). A 9 mm disc of actively growing fungal culture was placed near the edge of petri dishes containing Potato Dextrose Agar (PDA) medium. On the opposite side of the plate, actively growing bacterial cultures were streaked. Three replications were maintained for each treatment along with control. The plates were incubated at room temperature until the mycelium in the control plates fully covered the agar surface. The petri plates were incubated at room temperature for 7-10days and the pathogen growth was measured against the newer generation fungicide separately for each isolate on 7th day after incubation. The inhibition zone was determined by measuring the distance between fungal growth and bacterial colonies. The percentage of mycelial growth inhibition compared to the control was calculated using the formula proposed by Vincent (1947):

I= C-T/C

* *I* = Inhibition of mycelial growth over control
* *C* = Mycelial growth in control
* *T* = Mycelial growth in treatment

**RESULTS AND DISCUSSION**

**Survey for the occurrence of *Alternaria* leaf spot in Tomato**

Survey for the incidence of *Alternaria* leaf spot incited by *Alternaria solani* in commercially cultivated tomato varieties and hybrids revealed that, the incidence ranged from 27.22 PDI to 53.75 % of tomato-growing districts in Tamil Nadu (Table 1).

“The disease was observed in 60% of the fields surveyed and incidence ranged from 20% to 80% during irrespective of cultivars sown. Infected plants in the field showed symptoms on all aerial parts of the plant (leaves, stems, buds, and pods)” (M Sharma, 2013). Overall, fungal blight represents a major barrier to successful tomato cultivation, with yield losses varying from 15% to 100% (Mathur and Shekhawat, 1986; Panthee and Chen, 2010). This versatile pathogen infects various plant parts, including leaves, stems, petioles, twigs, and fruits, leading to defoliation, twig dieback, and premature fruit drop, ultimately reducing yield by 30-65% (Saha and Das, 2013).

**Symptomatology**

**Tomato early blight**

*Alternaria* leaf spot, caused by *Alternaria solani*, affects various parts of the plant, leading to defoliation, drying of twigs and premature fruit drop under favorable conditions. The initial symptoms on the leaves present as small black to brown lesions measuring 1 to 2 mm, which expand to form concentric rings often encircled by a yellow halo under optimal conditions. Lesions larger than 10 mm frequently display dark pigmented concentric rings.

Mamgain *et al.* (2013) and Bessadat *et al*. (2017) reported that *Alternaria solani*, the causal agent of early blight, produces dark necrotic lesions with characteristic concentric rings, primarily on the older leaves of the plant. These symptoms are among the most common and diagnostic features of the disease. Under favorable conditions, these lesions expand and coalesce, leading to significant leaf damage and reduced photosynthetic area.

Govern (2011) also observed that early blight affects the stem, forming dark, slightly sunken lesions that develop into concentric ring patterns. These lesions typically appear just above the soil surface and can girdle the stem, disrupting the vascular system of the plant.

**Morphological Characterization of *Alternaria solani***

The fungi were isolated from *Alternaria* infected tomato leaves. Four different strains of *Alternaria solani* were isolated and maintained in PDA media. The collected samples were isolated using the single spore isolation technique. After the incubation period, the fungal colony was appeared due to the mycelial growth of the fungi. At the first stage, the mycelial was septate, branched, gray-brown to olivaceous with or without zonation. Colonies were appressed, spreading cottony to velvety in nature. The fungus produced obclavate or ovate or obpyriform to ellipsoidal conidia in chain, when the cultures were incubated at 23-25°C for 6 days on PDA. Conidia were brown to golden brown with 3-8 transverse and 1-2 longitudinal septations.

As reported by Kumar *et al*. (2014), some *A. solani* mycelium were black in color, while others exhibited yellow, greenish, or brownish-black pigmentation and also found that the length of the conidia varied from 150 to 300 μm, with a thickness range of 15 to 19 μm. Additionally, the color of the conidia ranged from dark muriform to pale golden and olivaceous brown. The conidia had between 9 and 11 transverse septa and 1 to 4 longitudinal septa.

Dhaval *et al. (*2023) has reported that the pathogen’s mycelium are septate, branched, hyaline and its darken in colour. It has single conidia which germinate by simple conidiophores. *A. solani* is a large-spored fungus that has horizontal and vertical septations.

***In vitro* screening of newer generation fungicides against *Alternaria solani***

By using eight newer generation fungicide used for screeningagainst *Alternaria solani* (Table 2.). The antagonistic effect of newer generation fungicides was assessed based on their ability to inhibit the mycelial growth of pathogen in dual culture. The effect of these antagonists on the mycelial growth of the pathogen was calculated and expressed as per cent inhibition (Table 3).

A total of eight newer generation fungicides were evaluated for their antagonistic activity against *Alternaria* leaf spot, caused by *Alternaria solani* (Fig 1.). From the results, it is evident that, among the newer generation fungicides tested, Azoxystrobin showed maximum inhibition (48.15%) against *Alternaria solani* followed by difenoconazole (42.73%), whereas least percent inhibition was recorded in case of Hexaconazole (21.78%).

Tofoli *et al.* (2003) assessed the efficacy of different fungicide groups in managing early blight in tomato and their impact on fruit yield. Their findings revealed that the best disease control, fruit quality, and yield improvement were achieved with Azoxystrobin, followed by Mancozeb and Chlorothalonil. Among the various fungicides tested, Azoxystrobin 11% + Tebuconazole 18.3% SC at 1000 ml/ha resulted in the lowest early blight incidence, recording a Percent Disease Index (PDI) of 17.14%, significantly lower than the untreated control, which showed 63.63% after the third foliar spray. This treatment also produced the highest fruit yield of 418.30 quintals per hectare, which was significantly higher than all other treatments (Palaiah *et al*., 2019).

**Conclusion**

The present study confirms that *Alternaria solani* is a significant constraint on tomato cultivation in Tamil Nadu, with field-observed PDI values ranging from 25% to 54%, similar findings in other major Indian tomato-growing regions and contributing to global yield losses of up to 100% (Sharma, 2013). Morphological assessments of five isolated strains (AS1–AS5) aligned with established descriptions Dhaval *et al.,* 2023), affirming the reliability of the identification methodology. *In vitro* screening revealed that Azoxystrobin (48.15% inhibition) and Difenoconazole (42.73%) outperformed other fungicides, suggesting the effectiveness of Group‑11 strobilurins and Group‑3 triazoles against early blight, consistent with the positive outcomes seen in previous studies involving azoxystrobin-based treatments (Tofoli *et al.,* 2003; Palaiah *et al*., 2019).

These findings underscore the importance of integrating high-efficacy fungicides like azoxystrobin into disease management programs. However, to ensure long-term effectiveness and resistance management, further research should focus on field evaluations, optimal dosing regimens, fungicide rotation strategies, and the incorporation of complementary approaches such as cultural practices and resistant tomato varieties. Such integrated strategies will be vital in sustaining tomato productivity while mitigating the impact of *A. solani* in tropical and subtropical agroecosystems.

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**Table 1. Survey for the occurrence of *Alternaria* leaf spot in Tomato**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S.No.** | **Village name** | **District** | **Latitude** | **longitude** | **PDI%** |
| 1 | Baburanyenpettai | Chengalpattu | 12.389134o | 79.743538o | 27.22d |
| 2 | Indamangalam | Dharamapuri | 12.204827 o | 78.218682 o | 53.75a |
| 3 | Kondampatti | Dharamapuri | 12.191312 o | 78.253268 o | 35.83b |
| 4 | Vellalapatti | Krishnagiri | 12.177653 o | 78.239665 o | 34.55c |
| **CD (p < 0.05)** | | | | | 3.916 |

**Table 2. Classification of newer generation fungicides based on FRAC group**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S.No** | **Fungicide name** | **Group name** | **Group number** | **Chemical formula** | **Dosage (a.i./ha)** |
|  | Pyraclostrobin 25% EC | Strobilurin | 11 | C₁₉H₁₈ClN₃O₄ | 150 g |
|  | Hexaconazole 5 EC | Triazole | 3 | C₁₆H₂₁Cl₂N₃O | 500 g |
|  | Azoxystrobin 25% SC | Strobilurin | 11 | C₂₂H₁₇N₃O₅ | 150 g |
|  | Trifloxystrobin 25% WG + Tebuconazole 50% 75 WG | Strobilurin, Triazole | 11, 3 | C₂₀H₁₇F₃N₂O₄, C₁₆H₂₂ClN₃O | 250–300 g |
|  | Tricyclazole 75% WP | Melanin biosynthesis inhibitors | 16 | C₉H₇N₃S | 225–300 g |
|  | Propineb 70% WP | Dithiocarbamates | M3 | C₅H₁₀N₂S₄Zn | 1000–1200 g |

**Table 3. *In vitro* screening of newer generation fungicides against *Alternaria solani***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S.No** | **Fungicide name** | **Mycelial growth** | **Inhibition zone** | **Inhibition over control** |
| 1 | Pyraclostrobin 25% EC | 67.67bc | 6.43c | 22.81e |
| 2 | Hexaconazole 5 EC | 69.67b | 4.26d | 21.78f |
| 3 | Azoxystrobin 25% SC | 45.00g | 17.66a | 48.15a |
| 4 | Trifloxystrobin 25% WG + Tebuconazole 50% 75 WG | 64.21c | 5.56c | 23.33e |
| 5 | Tricyclazole 75% WP | 65.66c | 5.66c | 24.63d |
| 6 | Propineb 70% WP | 54.66d | 11.67b | 36.99c |
| 7 | Difenoconazole | 49.00f | 14.00b | 42.73b |
| 8 | Control | 90.00a | 0.00e | 0.00g |
| **CD (p<0.05)** | | **2.34** | **2.75** | **1.20** |

**Fig 1**: ***In vitro* screening of newer generation fungicides against *Alternaria solani***