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Isolation and *in-vitro* evaluation of bioagents and fungicides for the management of early leaf blight of tomato (*Alternaria solani*)

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Abstract
Early blight of Tomato incited by *Alternaria solani is* considered to be the most devastating disease occurring all over the world. The present investigation on " Isolation and *In-vitro* Evaluation of Bioagents and Fungicides for the Management of Early Leaf Blight of Tomato (*Alternaria solani*)." was undertaken during 2024-2025 on the aspect *viz*., isolation, *in vitro* evaluation of bioagents, systemic, non-systemic and combi-product fungicides of fungicides and bioagents at Department of Plant Pathology, VNMKV, Parbhani. Typical early blight symptoms observed in the Department of Horticulture field at COA, Parbhani, included circular brown spots with concentric rings, which later coalesced, causing blighting, defoliation, stem cankers, and fruit rot under severe infection. The pathogen's identity was confirmed as *Alternaria solani* (Ellis and Martin) Jones and Grout based on symptoms, cultural and morphological features, microscopic examination on the susceptible Tomato local variety. *in vitro* evaluation of fungicides and bioagents revealed that the systemic fungicide Difenoconazole, the non-systemic fungicide Mancozeb and the combi-fungicides Azoxystrobin + Tebuconazole and Azoxystrobin + Difenoconazole showed the highest inhibition of mycelial growth (100%), with the least colony diameter (6.66 mm). Among bioagents, *Trichoderma harzianum* was most effective, completely inhibiting the mycelial growth (0.00 mm, 100% inhibition), followed by *T. asperellum* (16.00 mm & 82.21%).

Keywords: Early blight, tomato, *Alternaria solani*, bioagents, fungicides, management

Introduction
Tomato (*Lycopersicon esculentum* Mill.) is among the most widely cultivated and economically valuable vegetable crops in the world. Praised for its rich nutritional profile, Tomato is considered a "protective food" due to its abundance of essential nutrients like vitamins A, C, lycopene, minerals, amino acids, and fiber. These nutrients not only promote good health but also help prevent nutritional deficiencies. Globally, Tomato ranks second only to Potato in terms of vegetable production with its importance spanning both fresh consumption and food processing industries. Tomato originated in Central and South America and was introduced to India by the Portuguese around the year 1700 (Kale and Kale, 1994) [10]. Today, it is cultivated extensively across India with Bihar leading in production followed by Uttar Pradesh, Karnataka, Punjab and others. As of 2024, India recorded a production of approximately 21.89 million tonnes from 1.39 million hectares, achieving an average yield of 24.18 tonnes per hectare. In Maharashtra alone, tomato was grown on 55.2 thousand hectares, yielding over 1.33 million tonnes. Despite its high nutritional value, Tomato cultivation is threatened by several biotic stresses, especially fungal pathogens. Major fungal diseases include damping-off (*Pythium aphanidermatum*), late blight (*Phytophthora infestans*), fruit rot (*Alternaria alternata*), early blight (*Alternaria solani*), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*) and powdery mildew (*Leveillula taurica*). Other challenges include bacterial diseases like wilt and canker, nematodes like *Meloidogyne spp*. and viral diseases such as Tomato leaf curl and mosaic viruses. Among these, Early leaf blight caused by *Alternaria solani* is one of the most severe and economically damaging fungal diseases of Tomato.

It is both soil and air borne capable of infecting all above ground plant parts and causing up to 30% premature fruit drop (Walker, 1951) [26]. Early symptoms appear as small dark brown spots on older leaves. These lesions develop concentric rings, giving a "Target board" appearance and are surrounded by yellowing tissue. As the disease progresses leaves dry out and drop prematurely, reducing the plant ability to photosynthesize and ultimately lowering yield. The disease also affects stems and fruits, leading to dry, sunken lesions that can girdle stems or reduce fruit marketability. Yield losses from Early blight are staggering, ranging from 48% to 80% in countries like India, Canada, the USA, and Nigeria (Basu, 1974; Gwary & Nahunnarao, 1998) [2, 9]. In India, the disease was first documented by Butler and later reported across states like Tamil Nadu, Punjab and Madhya Pradesh. In Maharashtra, the disease is most severe during the rainy (*Kharif*) season with reported yield losses of 40-80% (Datar & Mayee, 1981) [5]. Peralta *et al*. (2005) [18] observed that *A. solani* affects all aerial parts of the plant. Saha and Das (2012) [21] quantified the impact stating that for every 1% rise in disease severity, yield loss increases by around 0.75 to 0.77 tonnes per hectare. A recent 2024 study on *Lycopersicon esculentum* reinforced these findings, indicating that tomato crops typically suffer from 2 to 5 fungal diseases simultaneously, with Early leaf blight being the most dominant during the rainy season. Disease incidence is generally higher in the monsoon compared to winter due to favourable moisture and Temperature conditions (Deshpande and Dhotre, 2017) [6].

Material and Methods

Methodology (Short Form with Paragraphs and Formulas)

The present investigation on *Alternaria solani* causing early blight in tomato (*Solanum lycopersicum* L.) was carried out during 2023 at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani. Infected leaf samples exhibiting typical early blight symptoms were collected from local tomato fields in Parbhani. The pathogen was isolated on Potato Dextrose Agar (PDA) medium under aseptic conditions. Pure cultures were obtained using the single hyphal tip method and identified based on colony morphology and microscopic characteristics of the mycelium and conidia, following standard descriptions. To confirm pathogenicity, a spore mycelial suspension of *A. solani* was prepared and sprayed onto healthy, susceptible tomato seedlings grown in sterilized earthen pots containing a 2:1:1 (w/w) mixture of soil, sand, and FYM. Inoculated plants were observed under screen house conditions for symptom development. Control plants were sprayed with sterile water to compare disease appearance.

For disease management studies, seven fungal bioagents were evaluated *in vitro* using the dual culture technique. Mycelial inhibition of the pathogen was calculated when the untreated control plates were fully colonized. The inhibition percentage was computed using the formula by Arora and Upadhyay.

The efficacy of systemic, non-systemic, and combi-product fungicides was evaluated using the poisoned food technique (Nene & Thapliyal, 1993) at concentrations of 500, 1000 ppm (for systemic), and 2000, and 2500 ppm (for non-systemic and combi-products). Growth inhibition was calculated using Vincent's (1927) formula. All experiments were laid out in Completely Randomized Design (CRD) with eight treatments (seven test agents plus untreated control) and three replications. Observations on radial mycelial growth and colony diameter were recorded periodically and analysed statistically.

Results

Present studies on the Early Leaf Blight disease of Tomato (*Lycopersicon esculentum* Mill) cause by the pathogen of [*Alternaria solani* (Ellis and Martin) Jones and Grout] was undertaken during *kharif*, 2024 at the Department of Plant Pathology, College of Agriculture, Parbhani, on the aspects *viz.*, symptomatology, isolation, *in-vitro* bio efficacy of fungicides, bioagents against *A. solani* causing blight disease of tomato. The results obtained on all these aspects are being presented here.

Isolation, and Identification of *Alternaria solani*

Symptomatology

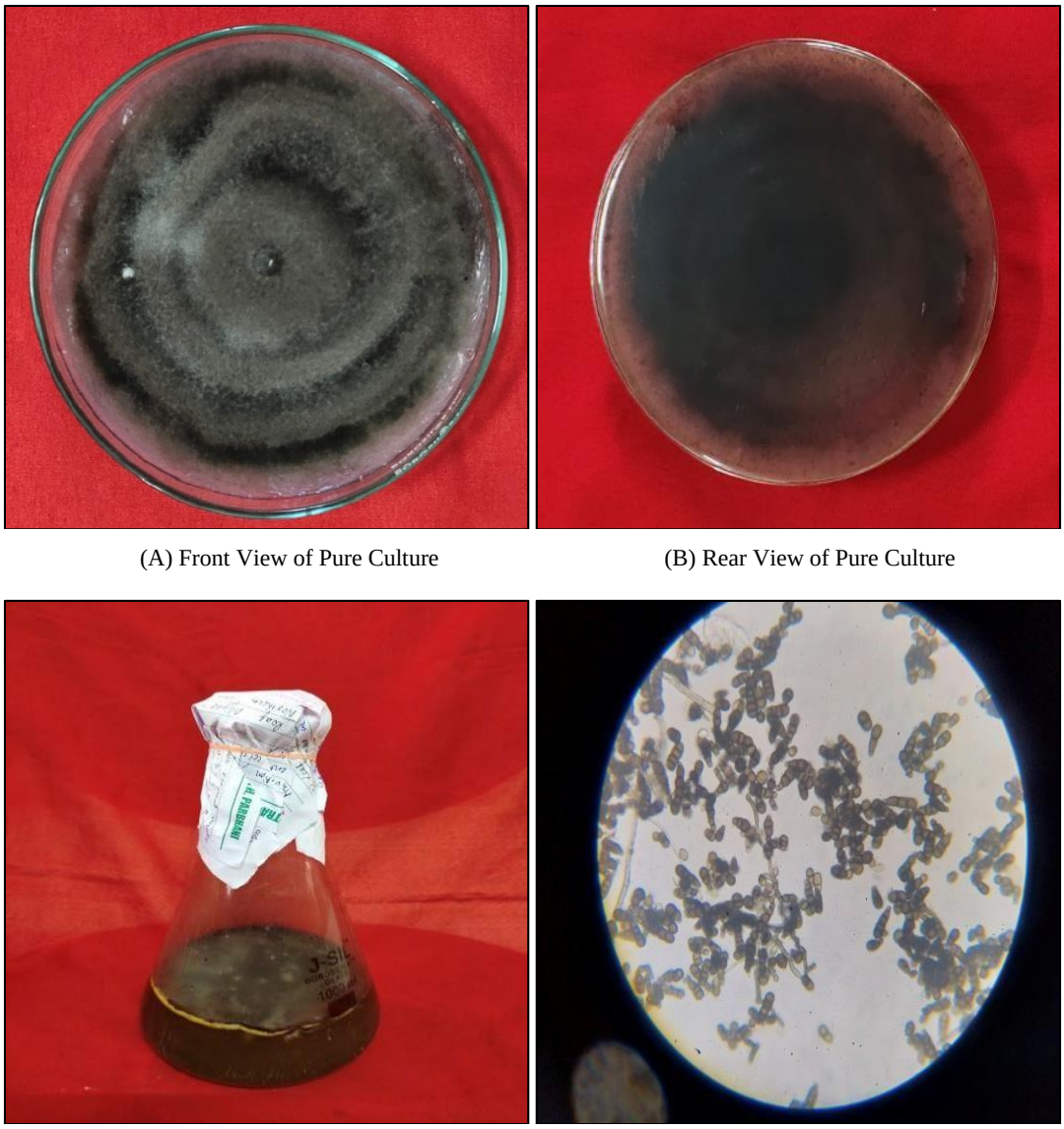
The first symptoms in addition to foliar symptoms, stem lesions were also observed as the disease progressed. These appeared as elongated, linear black streaks, typically starting near the nodes or lower stem region. Many of these stem lesions developed pale or greyish center, similar to the leaf spots, indicating systemic colonization by the pathogen. In advanced stages, multiple stem lesions coalesced and weakened the structural integrity of the plant, potentially resulting in lodging or stem breakage.

These observations provide clear evidence of the pathogenicity of *Alternaria solani* and align with the well documented symptomatology of early blight in tomato. The development of typical concentric lesions, chlorosis, defoliation and stem streaks are considered diagnostic features of this disease are critical for accurate field identification and subsequent disease management strategies.

Isolation and Purification

The pathogen was isolated from naturally infected tomato leaves showing typical early blight symptoms collected from the Department of Horticulture, College of Agriculture, VNMKV, Parbhani. Infected leaf segments were surface sterilized using 0.1% Naocl or 70% ethanol for 30-60 seconds, rinsed thrice in sterile distilled water, and transferred aseptically onto Potato Dextrose Agar (PDA) medium in Petri dishes.

Plates were incubated at 28 ± 2 °C under alternating light and dark conditions in a BOD incubator. Fungal growth was observed within 10-12 days. Colonies were initially white and cottony, turning dark brown to black with a leathery texture upon maturation typical of *A. solani*. Hyphal tip transfer was used for purification, and cultures were maintained on PDA by sub-culturing every 20 days. Mass multiplication was carried out in Potato Dextrose Broth (PDB) to obtain inoculum for further studies.



(A) Front View of Pure Culture (B) Rear View of Pure Culture

(C) Broth of *A. solani* (D) Microphotograph of *A. solani* conidia at 40X

Plate I: Isolation of *Alternaria solan*

Identification

The identification of *A. solani* was based on cultural characteristics on PDA and microscopic examination. Colonies transitioned from white to dark brown or black with flattened, leathery growth. Microscopic observations using lactophenol cotton blue staining revealed obclavate to ellipsoidal conidia, produced singly or in chains, with 3-7 transverse and 1-3 longitudinal septa. Conidia were dark brown and beaked at one or both ends. Conidiophores were septate, dark-colored and occasionally branched.

These morphological features confirmed the identity of the pathogen as *Alternaria solani* (Ellis and Martin). Comparable observations were reported by Alhussaen (2012) [1], who noted conidial dimensions ranging from 35-75 µm in length and 10-20 µm in width, with 2-7 transverse and 1-4 longitudinal septa, indicating variability among *A. solani* isolates.

Disease management strategies

Efficacy of bioagents against *Alternaria solani*

A total of seven bioagents (T_1-*Trichoderma asperellum*), (T_2-*Trichoderma harzianum*), (T_3-*Aspergillus niger*), (T_4-*Verticillium lecanii*), (T_5-*Nomuraea rileyi*), (T_6-*Beauveria bassiana*), and (T_7-*Metarhizium anisopliae*) were evaluated *in vitro* for their effectiveness against *Alternaria solani*, the pathogen responsible for Early leaf blight in Tomato.

The evaluation was conducted using the Dual Culture Technique (Arora and Upadhyay, 1978), with Potato Dextrose Agar (PDA) serving as the growth medium. This method allowed direct observation of the antagonistic interaction between each bioagent and the pathogen. The results of the experiment are summarized in Table 1.

Radial mycelial growth

Result revealed that, all seven bioagents tested exhibited antifungal activity against *A. solani* and were found to be effective in checking the mycelial growth of *Alternaria. solani* over untreated control (T_0).

Among the bioagents tested the radial mycelial growth of *A. solani* was ranged from 00.00 mm (T_2-*Trichoderma harzianum*) to 39.66 mm (T_6-*Beauveria bassiana*). The Treatment, (T_2-*Trichoderma harzianum***)** (00.00 mm) was found significantly superior to all other treatments including untreated control (T_0) with complete prohibition of mycelial growth. This was followed by the bioagents *viz*, (T_3-*Aspergillus niger*) (10.16 mm), (T_1-*Trichoderma asperellum*) (15.66 mm), (T_7-*Metarhizium anisopliae*) (19.66 mm) and (T_5-*Nomuraea rileyi*) (31.66 mm). The

bioagents (T_4-*Verticillium lecanii*) and (T_6-*Beauveria bassiana*) were recorded the maximum mycelial growth of *A. solani* i.e., 37.33 and 39.66 mm, respectively. All the treatments were statistically significant with each other including untreated control (T_0).

Mycelial inhibition

Result revealed that, all the seven bioagents tested exhibited antifungal activity against *A. solani* and significantly inhibited its growth, over untreated control (T_0).

Among the bioagents tested mycelial growth inhibition of *A. solani* was ranged from 55.93 percent (T_6-*Beauveria bassiana*) to 100.00 percent (T_2-*Trichoderma harzianum*). The Treatment, (T_2-*Trichoderma harzianum*) (100%) was found significantly superior to all other treatments including untreated control (T_0) with complete inhibition of mycelial growth. This was followed by the treatments, (T_3-*spergillus niger*) (88.71%), (T_1-*Trichoderma asperellum*) (82.60%), (T_7-*Metarhizium anisopliae*) (78.15%) and (T_5-*Nomuraea* rileyi) (64.82%). Whereas, the treatments (T_4-*Verticillium lecanii*) and (T_6-*Beauveria bassiana*) recorded the least mycelial inhibition of *A. solani* i.e., 58.52 and 55.93%, respectively.

Table 1: Efficacy of bioagents against *Alternaria solani*.

Tr. No.	Bioagents	Radial mycelial growth *(mm)	Percent inhibition
T_1	*Trichoderma asperellum*	15.66	82.60 (65.35)**
T_2	*Trichoderma harzianum*	00.00	100.00 (90.00)
T_3	*Aspergillus niger*	10.16	88.71 (70.37)
T_4	*Verticillium lecanii*	37.33	58.52 (49.91)
T_5	*Nomuraea rileyi*	31.66	64.82 (53.62)
T_6	*Beauveria bassiana*	39.66	55.93 (48.41)
T_7	*Metarhizium anisopliae*	19.66	78.15 (62.13)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E. (m)±		0.38	0.63
C.D.at 1%		1.06	1.95

* Mean of three replications.

**Figure in parenthesis are angular transformed values.

The results obtained are found agreed with the Ganie *et al*. (2013) [7] who evaluated biocontrol agents against *A. solani* under *in vitro* condition and reported that *Trichoderma harzianum* showed maximum mycelial growth inhibition (71.85%) which was followed by *Trichoderma viride* (65.93%) and *Trichoderma virens* (58.65%). Chohan *et al*. (2015) [4] proved that *Trichoderma harzianum* and *Trichoderma viride* were effective in inhibiting *A. solani* under *in vitro* condition. Singh *et al*. (2018) [22] reported that *Trichoderma harzianum* was most effective and showed highest inhibition of *A. solani* followed by *Trichoderma viride* and *Trichoderma koningii*. However, *Trichoderma hamatum* was least effective with highest mycelial growth. Naik *et al*. (2020) [15] observed that *Trichoderma harzianum* was most effective among the evaluated biocontrol agents. It showed highest inhibition (85.13%) which was followed by *Trichoderma viride* (80.67%) and *Pseudomonas fluorescens* (72.87%) while least inhibition (66.16%) was showed by *Trichoderma hamatum*.

Efficacy of systemic fungicides against *Alternaria solani*

A total of seven systemic fungicides *viz*., (T_1-Propiconazole), (T_2-Tebuconazole), (T_3-Azoxystrobin), (T_4-Carbendazim), (T_5-Thiophanate-methyl), (T_6-Difenoconazole) and (T_7-Pyraclostrobin) were tested at three different concentrations (500, 1000 ppm) *in vitro* against *Alternaria solani*, applying Poisoned Food Technique (Nene and Thapliyal, 1993) and using basal media as Potato Dextrose Agar (PDA). Table 2

Systemic fungicides at 500 ppm

Radial mycelial growth:

At 500 ppm concentrations, as shown in the Table 2 the radial mycelial growth of *A. solani* was ranged from 00.00 mm (T_1-Propiconazole), (T_2-Tebuconazole) and (T_6-Difenoconazole) to 39.50 mm (T_4-Carbendazim). The fungicides (T_1-Propiconazole), (T_2-Tebuconazole) and (T_6-Difenoconazole) arrested cent percent mycelial growth (00.00 mm), these treatments were statistically at par with each other and significantly superior over other treatments including untreated control (T_8). The next best fungicides with least mycelial growth were (T_5-Thiophanate-methyl) (14.50 mm) followed by (T_7-Pyraclostrobin) (18.50 mm) and (T_3-Azoxystrobin) (19.50 mm). Both treatments (T_7-Pyraclostrobin) and (T_3-Azoxystrobin) were statistically at par with each other. The fungicide (T_4-Carbendazim) recorded maximum mycelial growth of *A. solani i.e*., 39.50 mm.

Mycelial inhibition

At 500 ppm concentrations, as shown in the mycelial growth inhibition of *A. solani* was ranged from 55.92% (T_4-Carbendazim) to 100% (T_1-Propiconazole), (T_2-Tebuconazole) and (T_6-Difenoconazole). The fungicides (T_1-Propiconazole), (T_2-Tebuconazole) and (T_6-Difenoconazole) gave percent (100%) mycelial inhibition, these treatments were statistically at par with each other and significantly superior over other treatments including untreated control (T_8). The next best fungicides found were (T_5-Thiophanate-methyl) (83.14%) followed by (T_7-Pyraclostrobin) (78.70%) and (T_3-Azoxystrobin) (77.59%). The treatment (T_7-Pyraclostrobin) and (T_3-Azoxystrobin) both were statistically at par with each other. Whereas, the fungicide (T_4-Carbendazim) was found least effective with mycelial inhibition of 55.92 percent only**.**

Table 2: Effect of systemic fungicides on *Alternaria solani* at 500 ppm concentration

Tr. No.	Fungicides	Radial mycelial growth *(mm)	Percent inhibition
T_1	Propiconazole 25% EC	00.00	100.00 (90.00)**
T_2	Tebuconazole 25.9% EC	00.00	100.00 (90.00)
T_3	Azoxystrobin 23% SC	19.50	77.59 (62.26)
T_4	Carbendazim 50% WP	39.50	55.92 (48.51)
T_5	Thiophanate-methyl 70% WP	14.50	83.14 (66.33)
T_6	Difenoconazole 25% EC	00.00	100.00 (90.00)
T_7	Pyraclostrobin 20% WG	18.50	78.70 (63.04)
T_8	Control (untreated)	90.00	00.00 (00.00)
S.E. (m)±		0.18	0.97
C.D.at 1%		0.59	2.79

* Mean of three replications.
**Figure in parenthesis are angular transformed values.

Systemic fungicides at 1000 ppm
Radial mycelial growth
At 1000 ppm concentrations, as shown in the Table 2 the radial mycelial growth of *A. solani* was ranged from 00.00 mm (T_1-Propiconazole), (T_2-Tebuconazole) and (T_6-Difenoconazole) to 19.33 mm (T_4-Carbendazim). The fungicide (T_1-Propiconazole), (T_2-Tebuconazole) and (T_6-Difenoconazole) arrested cent percent mycelial growth (00.00 mm), these treatments were found statistically at par with each other and significantly superior over other treatments including untreated control (T_8). The next best fungicides with least mycelial growth recorded were (T_5-Thiophanate-methyl) (11.99 mm), (T_7-Pyraclostrobin) (12.88 mm) and (T_3-Azoxystrobin) (14.16). The fungicide (T_4-Carbendazim) recorded maximum mycelial growth of 19.33 mm at 1000 ppm concentrations.

Mycelial inhibition
At 1000 ppm concentrations, as shown in the mycelial growth inhibition of *A. solani* was ranged from 77.40% (T_4-Carbendazim) to 100% (T_1-Propiconazole), (T_2-Tebuconazole) and (T_6-Difenoconazole). The fungicide (T_1-Propiconazole), (T_2-Tebuconazole) and (T_6-Difenoconazole) gave cent percent (100%) mycelial inhibition over (T_8) untreated control (00.00%). These treatments (T_1, T_2 and T_6) were statistically at par with each other and significantly superior over other treatments including untreated control (T_8). The next best fungicides which recorded highest mycelial inhibition were (T_5-Thiophanate-methyl) (88.51%), (T_7-Pyraclostrobin) (85.55%) and (T_3-Azoxystrobin) (84.25%). Whereas, the fungicide (T_4-Carbendazim) was found least effective with minimum mycelial inhibition of 77.40 percent against *A. solani***.**

Table 3: Effect of systemic fungicides on *Alternaria solani* at 1000 ppm concentration

Tr. No.	Fungicides	Radial mycelial growth *(mm)	Percent inhibition
T_1	Propiconazole 25% EC	00.00	100.00 (90.00)**
T_2	Tebuconazole 25.9% EC	00.00	100.00 (90.00)
T_3	Azoxystrobin 23% SC	14.16	84.25 (66.59)
T_4	Carbendazim 50% WP	19.33	77.40 (61.59)
T_5	Thiophanate-methyl 70% WP	11.99	88.51 (70.16)
T_6	Difenoconazole 25% EC	00.00	100.00 (90.00)
T_7	Pyraclostrobin 20% WG	12.88	85.55 (67.63)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E. (m)±		0.19	1.20
C.D.at 1%		0.62	3.61

*Mean of three replications.
**Figure in parenthesis are angular transformed values.

The present findings are found similar with the Rani *et al*. (2017) [19] who evaluated nine fungicides at various concentrations *viz*. 10, 25, 50 and 100 ppm under *in vitro* condition against *A. solani* and reported that tebuconazole 25.9% EC was the most effective fungicide which showed highest mycelial growth inhibition (94.44%) at 100 ppm concentration.
They also reported that the efficacy of fungicides was significantly increased with the increase in the concentration. 28 At higher concentration more inhibition was observed than at lower concentration. Sreenivasulu *et al*. (2019) [23] reported that tebuconazole 25.9% EC @ 0.1% was most effective fungicide against *A. solani* showed 100 percent mycelial growth inhibition under *in vitro* condition. Ghule *et al*. (2021) [8] reported 100 percent mycelial growth inhibition of *A. solani* with difenoconazole, propiconazole, tebuconazole and propineb.

Efficacy of non-systemic fungicides against *Alternaria solani*
A total of seven non-systemic fungicides *viz*., Mancozeb (T_1), Chlorothalonil (T_2), Copper oxychloride (T_3), Propineb (T_4), Copper Hydroxide (T_5), Sulphur (T_6) and Zineb (T_7) were tested at three different concentrations (2000, 2500 ppm) *in vitro* against *Alternaria solani*, applying Poisoned Food Technique (Nene and Thapliyal, 1993) and using basal media as Potato Dextrose Agar (PDA). Effects of these fungicides on radial mycelial growth and inhibition was recorded and the results obtained are presented here. The results obtained are presented in Table 4 to 5.

Non-systemic fungicides at 2000 ppm
Radial mycelial growth
At 2000 ppm concentrations, as shown in the Table 4 the radial mycelial growth of *A. solani* was ranged from 00.00

mm (T_1-Mancozeb (T_1) and (T_4-Propineb) to 40.66 mm (T_3-Copper oxychloride). The fungicide (T_1-Mancozeb) (00.00 mm) and (T_4-Propineb) (00.00 mm) were significantly superior to all other treatments including untreated control (T_8) with complete prohibition of mycelial growth. The next best fungicides with significantly least mycelial growth recorded was (T_2-Chlorothalonil) (20.49 mm), followed by (T_5-Copper Hydroxide) (28.83 mm), (T_7-Zineb) (30.78 mm) and (T_6-Sulphur) (34.08 mm). The fungicide (T_3-Copper oxychloride) recorded the maximum mycelial growth of 40.66 mm against *A. solani*. All these treatments were statistically significant with each other and over untreated control (T_8) except (T_1-Mancozeb) and (T_4-Propineb) which were statistically at par with each other.

Table 4: Effect of non-systemic fungicides on *Alternaria solani* at 2000 ppm concentration

Tr. No.	Fungicides	Radial mycelial growth *(mm)	Percent inhibition
T_1	Mancozeb 75% WP	00.00	100.00 (90.00)**
T_2	Chlorothalonil 75% WP	20.49	74.25 (59.48)
T_3	Copper oxychloride 50% WP	40.66	53.33 (46.89)
T_4	Propineb 70%WP	00.00	100.00 (90.00)
T_5	Copper Hydroxide 53.8% DF	28.83	66.84 (54.80)
T_6	Sulphur 80% WDG	34.08	58.51 (49.88)
T_7	Zineb 75% WP	30.78	62.95 (52.48)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m)±		1.15	1.66
C.D.at 1%		3.64	4.38

* Mean of three replications.
**Figure in parenthesis are angular transformed values.

Mycelial inhibition

At 2000 ppm concentrations, as shown in the mycelial growth inhibition of *A. solani* was ranged from 53.33% (T_3-Copper oxychloride) to 100% (T_1-Mancozeb) and (T_4-Propineb). The fungicide (T_1-Mancozeb) (100.00%) and (T_4-Propineb) (100.00%) were significantly superior to all other treatments including untreated control (T_8) with complete inhibition of mycelial growth. The next best fungicide with significantly highest mycelial growth inhibition recorded was (T_2-Chlorothalonil) (74.25%), followed by (T_5-Copper Hydroxide) (66.84%), (T_7-Zineb)(62.95%) and (T_6-Sulphur) (58.51%). The fungicide (T_3-Copper oxychloride) found less effective with minimum mycelial inhibition of 53.33 percent over untreated control (T_8) 00.00 percent.

Non-systemic fungicides at 2500 ppm

Radial mycelial growth

At 2500 ppm concentrations, as shown in the Table 5 the radial mycelial growth of *A. solani* was ranged from 00.00 mm (T_1-Mancozeb) and (T_4-Propineb) to 30.00 mm (T_3-Copper oxychloride). The fungicide (T_1) Mancozeb (00.00 mm) and (T_4) Propineb (00.00 mm) was significantly superior to all other treatments including untreated control (T_8) with complete prohibition of mycelial growth. The next best fungicide with significantly least mycelial growth recorded was (T_2-Chlorothalonil) (19.50 mm), followed by (T_5-Copper Hydroxide) (21.83 mm), (T_7-Zineb) (23.33 mm), (T_6-Sulphur) (27.66 mm) and. The fungicide (T_3-Copper oxychloride) recorded the maximum mycelial growth of 30.00 mm against *A. solani*.

Mycelial inhibition

At 2500 ppm concentrations, as shown in the mycelial growth inhibition of *A. solani* was ranged from 66.66% (Copper oxychloride) (T_3) to 100% (T_1-Mancozeb) and (T_4-Propineb). The fungicide (T_1-Mancozeb) (100.00%) and (T_4-Propineb) (100.00%) was significantly superior to all other treatments including untreated control (T_8) with complete inhibition of mycelial growth. The next best fungicide with significantly highest mycelial growth inhibition recorded was (T_2-Chlorothalonil) (78.33%), followed by (T_5-Copper Hydroxide) (75.73%), (T_7-Zineb) (74.06%) and (T_6-Sulphur) (69.25%). The fungicide (T_3-Copper oxychloride) found less effective with minimum mycelial inhibition of 66.66 percent over untreated control (T_8) 00.00 percent.

Table 5: Effect of non-systemic fungicides on *Alternaria solani* at 2500 ppm concentration

Tr. No.	Fungicides	Radial mycelial growth *(mm)	Percent inhibition
T_1	Mancozeb 75% WP	00.00	100.0 (90.00)**
T_2	Chlorothalonil 75% WP	19.50	78.33 (62.23)
T_3	Copper oxychloride 50% WP	30.00	66.66 (54.71)
T_4	Propineb 70%WP	00.00	100.00 (90.00)
T_5	Copper Hydroxide 53.8% DF	21.83	75.73 (60.46)
T_6	Sulphur 80% WDG	27.66	69.25 56.30)
T_7	Zineb 75% WP	23.33	74.06 (59.36)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m)±		0.14	0.16
C.D.at 1%		0.43	0.48

* Mean of three replications.
**Figure in parenthesis are angular transformed values.

The similar results were earlier reported by Khan *et al*. (2007) [11], who reported that cent percent mycelial growth inhibition of *Alternaria brassicae* was reported with Mancozeb @ 500 ppm concentration. Some of other researchers also reported similar finding such as Mishra *et al*. (2009) [12].

Efficacy of combi fungicides against *Alternaria solani*

A total of seven combi fungicides *viz*., (T_1-Thiophanate-methyl + Pyraclostrobin), (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole), (T_4-Fluxapyroxad + Pyraclostrobin), (T_5-Pyraclostrobin + Epoxyconazole), (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam) and Carbendazim + Mancozeb) were tested at three different concentrations (2000, 2500 ppm) against *A. solani*, applying Poisoned Food Technique (Nene and Thapliyal, 1993) and using basal media as Potato Dextrose Agar (PDA). Effects of these fungicides on radial mycelial growth and inhibition was recorded and the results obtained are presented here. The results obtained are presented in Table 6 to 7.

Combi fungicides at 2000 ppm

Radial mycelial growth

At 2000 ppm concentrations, as shown in the Table 6 the radial mycelial growth of *A. solani* was ranged from 00.00 mm (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam) to 8.16 mm (T_7-Carbendazim + Mancozeb). The combi fungicide (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam) recorded the minimum mycelial growth of (00.00) mm which was statistically at par with each other and were significant over rest of the treatments including untreated control (T_0). The next best combi fungicides with significantly least mycelial growth recorded were (T_4-Fluxapyroxad + Pyraclostrobin) (6.99 mm), followed by (T_5-Pyraclostrobin + Epoxiconazole) (7.83 mm). (T_1-Thiophanate-methyl + Pyraclostrobin) and (T_7-Carbendazim + Mancozeb) were statistically at par with each other and recorded a mycelial growth of 7.33 and 8.16 mm, respectively.

Table 6: Effect of combi-product fungicides on *Alternaria solani* at 2000 ppm concentration

Tr. No.	Fungicides	Radial mycelial growth *(mm)	Percent inhibition
T_1	Thiophanate-methyl 45% + Pyraclostrobin 5% (50% FS)	7.33	91.85 (73.41) **
T_2	Azoxystrobin 11% + Tebuconazole 18.3% (29.3% SC)	00.00	100.00 (90.00)
T_3	Azoxystrobin 18.2% + Difenoconazole 11.4% (29.6% SC)	00.00	100.00 (90.00)
T_4	Fluxapyroxad 25% + Pyraclostrobin 25% (50% SC)	06.99	92.23 (73.81)
T_5	Pyraclostrobin 13.3% + Epoxyconazole 5% (18.3%SE)	07.83	91.30 (72.85)
T_6	Azoxystrobin 2.5% + Thiophanate Methyl 11.25% +Thiamethoxam 25% (38.75% FS)	00.00	100.00 (90.00)
T_7	Carbendazim 12% + Mancozeb 63% (75% WP)	08.16	90.09 (71.65)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m)±		0.18	0.39
C.D.at 1%		0.59	1.2

* Mean of three replications.

**Figure in parenthesis are angular transformed values.

Mycelial inhibition

At 2000 ppm concentrations, as shown in the mycelial growth inhibition in combi fungicides was ranged from 90.09% (T_7-Carbendazim + Mancozeb) to 100% (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam). The fungicide (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam) were most superior thereby inhibiting the cent percent (100%) mycelial inhibition of *A. solani*. These treatments (T_2, T_3 and T_6) were statistically at par with each other and were significant over rest of the treatments including untreated control (T_0). The next best combi fungicides with significantly highest mycelial growth inhibition recorded were (T_4-Fluxapyroxad + Pyraclostrobin) (92.23%), followed by (T_5-Pyraclostrobin + Epoxiconazole) (91.30%) and (T_1-Thiophanate-methyl + Pyraclostrobin) (91.85%). The treatment (T_7-Carbendazim + Mancozeb) (90.09%), recorded the least mycelial growth inhibition of *A. solani* (90.09%) at 2000 ppm concentrations.

Combi fungicides at 2500 ppm

Radial mycelial growth

At 2500 ppm concentrations, as shown in the Table 7 radial mycelial growth of *A. solani* was ranged from 00.00 mm (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole), (T_4-Fluxapyroxad + Pyraclostrobin) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam) to 7.16 mm (T_7-Carbendazim + Mancozeb). The fungicide (T_2-Azoxystrobin + Tebuconazole) recorded the minimum mycelial growth of 00.00 mm which was statistically at par with (T_3-Azoxystrobin + Difenoconazole), (T_4-Fluxapyroxad + Pyraclostrobin) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam) (00.00 mm). These treatments (T_2, T_3, T_4 and T_6) were statistically at par with each other and significant over rest of the treatments including untreated control (T_0). The next best combi fungicides with significantly least mycelial growth recorded were (T_5-Pyraclostrobin + Epoxiconazole) (6.16 mm), followed by (T_1-Thiophanate-methyl + Pyraclostrobin) (6.66 mm) and (T_7-Carbendazim + Mancozeb) (7.16 mm), respectively. The treatment (T_7) recorded the highest radial mycelial growth of 7.16 mm at 2500 ppm concentration and was statistically significant over rest of the treatments including untreated control (T_8) which recorded full growth (90.00 mm).

Mycelial inhibition

At 2500 ppm concentrations, as shown in the mycelial growth inhibition of *A. solani* was ranged from 92.04% (T_7-Carbendazim + MancozebT_7) to 100% (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole), (T_4-Fluxapyroxad + Pyraclostrobin) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam). All these treatments

were statistically significant with each other except T_2, T_3, T_4 and T_6, which were at par with each other. The fungicide (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole), (T_4-Fluxapyroxad + Pyraclostrobin) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam) were most superior thereby inhibiting the percent (100%) mycelial inhibition of *A. solani*. The next best fungicides with significantly highest mycelial growth inhibition recorded were (T_5-Pyraclostrobin + Epoxiconazole) (93.15%), followed by (T_1-Thiophanate-methyl + Pyraclostrobin) (92.60%) and (T_7-Carbendazim + Mancozeb) (92.04%), respectively. Treatments (T_2-Azoxystrobin + Tebuconazole), (T_3-Azoxystrobin + Difenoconazole), (T_4-Fluxapyroxad + Pyraclostrobin) and (T_6-Azoxystrobin + Thiophanate Methyl + Thiamethoxam) were statistically at par with each other and significant over rest of the treatments.

The similar results were reported by earlier workers. Pamir *et al*. (2024) [17] studied the effect of combi product fungicides against *Alternaria solani* of Tomato and they reported that Azoxystrobin 18.2% + Difenoconazole 11.4% (29.6% SC) at 0.1 percent concentration and Azoxystrobin 11% + Tebuconazole 18.3% (29.3% SC) at 0.2 percent concentration were found to be most effective and significantly superior over rest of the combi product fungicides. Similarly, Surekha *et al*. (2024) [24] reported that Captan 70% + Hexaconazole 5% (75% WP), Tebuconazole 50% + Trifloxystrobin 25% (75% WG) and Azoxystrobin 18.2% + Difenoconazole 11.4% (29.26% SC) were significantly superior than other combi-product fungicides against *Alternaria solani* of potato.

Table 7: Effect of combi-product fungicides on *Alternaria solani* at 2500 ppm concentration

Tr. No.	Fungicides	Radial mycelial growth *(mm)	Percent inhibition
T_1	Thiophanate-methyl 45% + Pyraclostrobin 5% (50% FS)	06.66	92.60 (74.21)**
T_2	Azoxystrobin 11% + Tebuconazole 18.3% (29.3% SC)	00.00	100.00 (90.00)
T_3	Azoxystrobin 18.2% + Difenoconazole 11.4% (29.6% SC)	00.00	100.00 (90.00)
T_4	Fluxapyroxad 25% + Pyraclostrobin 25% (50% SC)	00.00	100.00 (90.00)
T_5	Pyraclostrobin 13.3% + Epoxyconazole 5% (18.3%SE)	06.16	93.15 (74.83)
T_6	Azoxystrobin 2.5% + Thiophanate Methyl 11.25% +Thiamethoxam 25% (38.75% FS)	00.00	100.00 (90.00)
T_7	Carbendazim 12% + Mancozeb 63% (75% WP)	07.16	92.04 (73.61)
T_0	Control (untreated)	90.00	00.00 (00.00)
S.E.(m)±		0.28	0.39
C.D.at 1%		0.86	1.22

* Mean of three replications.
**Figure in parenthesis are angular transformed values.

Conclusion

The present investigation successfully isolated, identified, and confirmed the pathogenicity of *Alternaria solani*, the causal agent of early blight in tomato. The pathogen was effectively cultured and maintained on PDA, and its disease symptoms were consistently reproduced under controlled conditions. *in vitro* evaluations revealed significant variability in the efficacy of different fungal bioagents and fungicides against the pathogen. Among bioagents, *Trichoderma* spp. showed the highest antagonistic activity. Several systemic, non-systemic, and combi-product fungicides also demonstrated promising levels of mycelial growth inhibition at varying concentrations. These findings provide a strong foundation for integrated disease management strategies and highlight effective control options for early blight in tomato under laboratory conditions. Field validation is essential to confirm their practical efficacy and sustainability under diverse agro-climatic conditions.

References

1. Alhussaen KM. Morphological and physiological characterization of *Alternaria solani* isolated from tomato in Jordan Valley. Research Journal of Biological Sciences. 2012;7(8):316-319.
2. Basu PK. Measuring early blight, its progress and influence on fruit losses in nine tomato cultivars. Canadian Plant Disease Survey. 1974;54:45-51.
3. Butler EJ. Fungi and diseases in plants. Memoirs of the Department of Agriculture in India-Botanical Series. 1918;7:1-160.
4. Chohan S, Perveen R, Mehmood MA, Naz N, Akram N. Morphophysiological studies, management and screening of tomato germplasm against *Alternaria solani* the causal agent of tomato early blight. International Journal of Agriculture and Biology. 2015;17(1):111-118.
5. Datar VV, Mayee CD. Assessment of losses in tomato yield due to early blight. Indian Phytopathology. 1981;34(1):191-195.
6. Deshpande AS, Dhotre DV. Fungal diseases of tomato and their occurrence in different seasons. International Journal of Current Microbiology and Applied Sciences. 2017;6(5):1437-1442. doi:10.20546/ijcmas.2017.605.156
7. Ganie SA, Ghani MY, Nissar Q, Jabeen N, Ahanger FA. Status and symptomatology of early blight (*Alternaria solani*) of potato (*Solanum tuberosum* L.) in Kashmir valley. African Journal of Microbiology Research. 2013;8(41):5104-5115.
8. Ghule PR, Lohate SR, Vavre KB, Bhagat AA. *in vitro* management of *Alternaria solani* through fungicides, bioagents and botanicals. The Pharma Innovation Journal. 2021;10(10):2320-2324.
9. Gwary DM, Nahunnarao H. Epiphytotics of early blight of tomatoes in North-Eastern Nigeria. Crop Protection. 1998;17:619-629.
10. Kale PN, Kale SP. Bhajipala Utpadan (Vegetable Production). Pune: Continental Publication Company; 1994. p. 29-30.
11. Khan MM, Khan RU, Mohiddin FA. Studies on the cost-effective management of *Alternaria* blight of

rapeseed-mustard (*Brassica* spp.). Phytopathologia Mediterranea. 2007;46:201-206.
12. Korbu AM. Studies on early blight of tomato incited by *Alternaria solani* (Ellis and Martin) Jones and Grout [MSc thesis]. Parbhani, Maharashtra: Marathwada Agricultural University; 2009.
13. Kumar V, Haldar S, Pandey K, Singh R, Singh A, Singh PC. Cultural, morphological, pathogenic, and molecular variability amongst tomato isolates of *Alternaria solani* in India. World Journal of Microbiology and Biotechnology. 2008;24(7):1003-1009.
14. Mishra PK, Rai RK, Saha S, Pandey KK, Singh RP, Rai AB. Effect of fungicides, botanicals and bioagent against leaf spot of cauliflower caused by *Alternaria brassicae* (Berk.) Sacc. Vegetable Science. 2009;36(3):356-359.
15. Naik SC, Narute TK, Narute TT, Khaire PB. *in vitro* efficacy of biocontrol agents against *Alternaria solani* (early blight of tomato). Journal of Pharmacognosy and Phytochemistry. 2020;9(5):550-552.
16. Nikam PS. Studies based on symptomatology, cultural and morphological characteristics, microscopic observations of *Alternaria solani* [MSc thesis]. 2013.
17. Pamir, Patil PV, Kulkarni S, Sridevi O, Hosamani RM. *in vitro* efficacy of fungicides against *Alternaria solani*, the incitant of early blight of tomato. International Journal of Advanced Biochemistry Research. 2024;8(5):287-292.
18. Peralta IE, Knapp S, Spooner DM. New species of wild tomatoes (*Solanum* section *Lycopersicon*: Solanaceae) from Northern Peru. Systematic Botany. 2005;30:424-434.
19. Rani S, Singh R, Gupta S. Development of integrated disease management module for early blight of tomato in Jammu. Journal of Pharmacognosy and Phytochemistry. 2017;6(2):268-273.
20. Rotem J. The genus *Alternaria*: biology, epidemiology, and pathogenicity. St. Paul, MN: APS Press; 1994.
21. Saha A, Das S. Estimation of crop loss due to early blight disease of tomato. Journal of Plant Pathology. 2012;94(2):407-410.
22. Singh VP, Khan RU, Pathak D. *in vitro* evaluation of fungicides, biocontrol agents and plant extracts against early blight of tomato caused by *Alternaria solani* (Ellis and Martin) Jones and Grout. International Journal of Plant Protection. 2018;11(1):102-108.
23. Sreenivasulu R, Surya Prakash Reddy M, Tomar DS, Subhash Sri Sanjay M, Bharath Bushan Reddy. Managing of early blight of tomato caused by *Alternaria solani* through fungicides and bioagents. International Journal of Current Microbiology and Applied Sciences. 2019;8(6):1442-1452.
24. Surekha SR, Kulkarni VR, Rao MSL, Shashidhar TR. Evaluation of different fungicides against *Alternaria solani* causing early blight disease of potato under *in vitro* conditions. Journal of Farm Sciences. 2024;37(1):20-24.
25. Thippeswamy B, Krishnappa M, Chakravarthy CN, Sathisha AM, Jyothi SU, Kumar V. Pathogenicity and management of leaf spot in brinjal caused by *Alternaria solani*. Indian Phytopathology. 2006;59(4):475-481.
26. Walker JC. Diseases of Vegetable Crops. New York: McGraw Hill Book Company; 1951. p. 470-475.