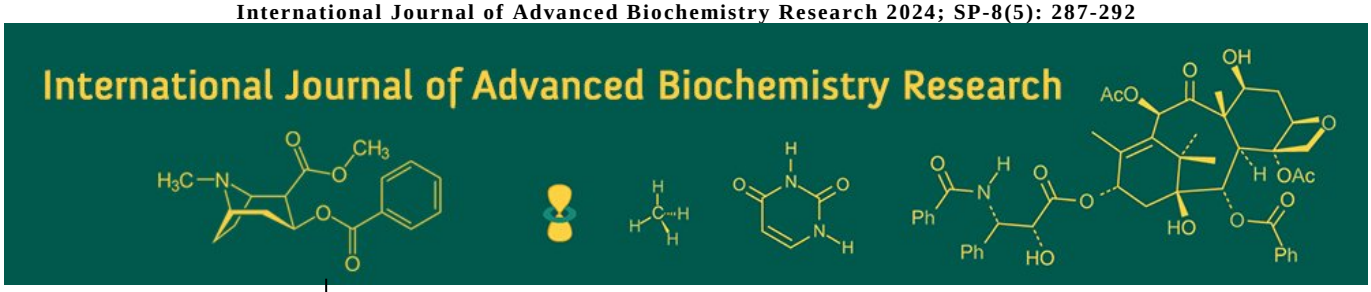

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In vitro efficacy of fungicides against *Alternaria solani*, the incitant of early blight of tomato

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Abstract
Tomato (*Solanum lycopersicum* L.) is an important and widely grown vegetable crop after potato, it is infected by number of diseases throughout their growing season. Among these diseases, early blight caused by *Alternaria solani* is the most destructive and threatening in all over the world, causing yield losses up to 80 percent resulting in a drastic reduction in the quantity and quality of fruit yield of tomato. In the present study, four non systemic fungicides at 0.1, 0.2 and 0.25 percent, six systemic fungicides at 0.025, 0.05 and 0.1 percent and ten combi product fungicides at 0.1, 0.2 and 0.3 percent concentration were tested *in vitro* against *Alternaria solani* by following poisoned food technique. Mancozeb 75% WP, difenoconazole 25% EC and (azoxystrobin 18.2% + difenoconazole 11.4%) 29.6% SC, among non-systemic, systemic and combi product fungicides, respectively inhibited maximum mycelial growth of the fungus and inhibition of mycelial growth was increased with increase in concentrations in all the tested fungicides.

Keywords: Poison food technique, mancozeb, difenoconazole, (azoxystrobin + difenoconazole)

Introduction
Tomato (*Solanum lycopersicum* L.) is the second most important and widely grown remunerative solanaceous vegetable crop after potato (Pritesh and Subramanian, 2011; Hadian *et al*., 2011) [14, 9]. Tomato is an important source of nutrients such as vitamin A, B, C and E, as well as proteins, carbohydrates and fibers. It is known to be free of cholesterol and contain lycopene, a potent antioxidant found in tomatoes, known to prevent cancers and protect against the harmful effects of free radicals that can degrade various bodily functions. (Sgherri *et al*., 2008) [15]. China leads in tomato production followed by India and the USA. It was perhaps introduced to India by the Portuguese during 1700 (Kale and Kale, 1994) [10]. In India, it is cultivated in an area of 840 thousand hectares with a production of 20.33 mt and with a productivity of 24.20 t/h. The major tomato growing states in the country include Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Odisha, West Bengal and Maharashtra. (www.Indiastat.com). Tomato crop is infected by several diseases throughout their growing season. Among the biotic causes, fungi are the most important ones which are responsible for many diseases such as early blight (*Alternaria solani* (Ellis and Martin) Jones and grout), late blight (*Phytophthora infestance* (Mont) De Bary.), damping off (*Pythium aphanidermatum* (Edson) Fitzp.), Septoria leaf spot (*Septoria lycopersici* Speg.), Fusarium wilt (*Fusarium oxysporium* f. sp. *Lycopersici* (Sacc.) Snyder and Hansen.), powdery mildew (*Leveillula taurica* (Lev.) G. Arnaud.) and buck eye rot (*Phytophthora* spp.) (Bost, 2013) [4].
Among the fungal diseases, the early blight caused by *Alternaria solani* is the most destructive and threatening, causing yield losses up to 80 per cent (Datar and Mayee, 1981, Chandravanshi *et al*., 1994, Balanchard, 1992, Gomaa, 2001, Abdel-Sayed, 2006 and Abada *et al*., 2008) [7, 5, 3, 8, 2, 1] resulting in a drastic reduction in the quantity and quality of fruit yield of tomato. The expenses associated with early blight management estimated around $32 million for tomatoes and approximately $45 million for potatoes (Kemmitt, 2002) [11]. While utilizing resistant genotypes may be considered as a promising alternative but there is lack of suitable tomato genotype resistant to *Alternaria solani* as indicated by Chowdappa *et al*. (2013) [6] and Moore *et al*. (2006) [12].

Consequently, chemical management with fungicides can be one of the effective and more popular methods due to their quick results, especially in the absence of resistant genotypes. Hence, different fungicides were evaluated under *in vitro* conditions to minimize the loss caused by the pathogen.

Materials and Methods

The present investigation was carried out during 2022 at the Department of Plant Pathology, College of Agriculture, Dharwad, Karnataka. The efficacy of four non systemic fungicides at 0.1, 0.2 and 0.25 percent, six systemic fungicides at 0.025, 0.05 and 0.1 percent and ten combi product fungicides at 0.1, 0.2 and 0.3 percent concentration were tested *in vitro* against *Alternaria solani* by following poisoned food technique (Nene and Thapliyal, 1982) [13]. The PDA medium was prepared and melted. The fungicidal suspension was added to the melted media to obtain the required concentrations. About 20 ml of poisoned medium was poured in each sterilized Petri plates. Suitable check was maintained without addition of fungicides. Eight mm mycelial disc was taken from the periphery of the colony and placed in the centre of Petri plate and incubated at 28 ± 1 °C. The experiment was planned with completely randomized design (CRD) and required number of replications were maintained for each treatment. The data on the percent mycelial inhibition of the pathogen was also converted into angular values and analyzed statistically. The diameter of the colony was measured after reaching maximum growth in control plates. The percent growth inhibition was calculated by using the formula given by Vincent (1947) [18] as follows,

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

Where,
PI = Percent inhibition of mycelial growth
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Results and Discussion

All the fungicides evaluated were significantly superior over the control with respect to percent mycelial inhibition. Among the non-systemic fungicides tested at three concentrations (0.1, 0.2 and 0.25%), maximum percent mycelial inhibition was recorded in treatments involving mancozeb 75% WP at all the three concentrations (73.50, 78.87 and 81.75%) which was found significantly superior over rest of the treatments followed by propineb 70% WP at 0.1 percent (68%), 0.2 percent (68.63%) and at 0.25 percent with 76.63 percent of mycelial growth inhibition. The least inhibition of mycelial growth was recorded in chlorothalonil 75% WP at 0.1 percent (55.00%). Irrespective of concentrations of fungicides tested, the treatment involving mancozeb 75% WP recorded maximum mean percent mycelial inhibition (78.04%) followed by propineb 70% WP (71.08%) and least percent mycelial inhibition was recorded in chlorothalonil 75% WP (59.21%) (Table 1 and Plate 1). Similarly, Singh *et al*. (2018) [16] tested the efficacy of different fungicides *in vitro* against early blight of tomato, among the non-systemic fungecides; mancozeb was effective at its all concentrations but recorded 100 percent inhibition only at its higher concentration of 400 ppm. Mancozeb disrupts enzymatic activities within fungal cells by targeting the sulfhydryl groups of amino acids, leading to interference in lipid metabolism, respiration and adenosine triphosphate production. This could be the probable reason for the inhibition observed in the growth of the test fungus.

Among systemic fungicides at 0.025 percent concentration, the highest percent (78.54) inhibition was noticed in difenoconazole 25% EC which was on par with hexaconazole 5% EC (77.08%). Least inhibition was noticed in thiophanate methyl 70% WP (17.71%) followed by validamycin 3% L (36.88%). At 0.05 percent concentration, 82.50 and 81.25 percent inhibition was recorded in difenoconazole 25% EC and hexaconazole 5% EC, respectively which are significantly superior over rest of the fungicides. Propiconazole and tebuconazole 25.9% EC were found next best and inhibited mycelial growth by 72.71 percent and 72.29 percent, respectively and were on par with each other. Least inhibition was recorded in thiophanate methyl (51.04%) (Table 2 and Plate 2).

Tebuconazole 25.9% EC recorded 85.83 percent inhibition of mycelial growth at 0.1% concentration which was on par with difenoconazole 25% EC which has recorded 85.63 percent inhibition. Least inhibition of 59.79 percent was recorded in thiophanate methyl. Irrespective of concentrations tested, difenoconazole 25% EC recorded highest inhibition of mycelial growth (82.22%) and least mycelial growth inhibition was observed in thiophanate methyl (42.85%). Similar observations were made by Pondkule (2020) [17] who assessed different fungicides at various concentrations against *A. solani* under *in vitro* conditions. Triazoles represent a potent category of fungicides with robust inhibition of ergosterol synthesis. This inhibition occurs through blocking the cytochrome P450-dependent enzyme, C-14 alpha de-methylase, essential for converting lanosterol to ergosterol. Insufficient production of normal sterols slows or stops the fungal growth, thus preventing further infection or invasion of host tissues.

Among ten combi product fungicides (Table 3 and Plate 3) maximum inhibition of mycelial growth (73.96%) was noticed in (azoxystrobin 18.2% + difenoconazole 11.4%) 29.6% SC at 0.1 percent concentration which was found to be most effective and significantly superior over rest of the combi product fungicides. Next best was (tricyclazole 18% + mancozeb 62%) 80% WP (72.50%). least inhibition was observed in (flusilozole 12.5% + carbendazim 25%) 37.5% EC (64.79%). At 0.2 percent concentration maximum inhibition was noticed in (azoxystrobin 11% + tebuconazole 18.3%) 29.3% SC (81.67%) which was significantly superior over other combi product fungicides. Next best was (captan 70% + hexaconazole 5%) 75% WP (80.00%) which was on par with (metalaxyl 8% + mancozeb 64%) 72% WP (78.96%) and (tricyclazole 18% + mancozeb 62%) 80% WP (79.9%). Significantly least inhibition was recorded in (zineb 68% + hexaconazole 4%) 72% WP and (carbendazim 12% + mancozeb 63%) 75% WP (68.13%). At 0.3 percent concentration, (azoxystrobin 18.2% + difenoconazole 11.4%) 29.6% SC (84.17%) was found significantly superior over other combi product fungicides, which was on par with (tebuconazole 50% + trifloxystrobin 25%) 75% WG (82.71%), (captan 70% + hexaconazole 5%) 75% WP (83.13%) and (azoxystrobin 11% + tebuconazole 18.3%)

29.3% SC (83.75%). The least inhibition was noticed in (zineb 68% + hexaconazole 4%) 72% WP (72.29%) and it was on par with (carbendazim 12% + mancozeb 63%) 75% WP (72.92%). Irrespective of fungicide concentration (azoxystrobin 18.2% + difenoconazole 11.4%) 29.6% SC (78.75%) was found best in inhibiting mycelial growth of *Alternaria solani* and remain on par with (metalaxyl 8% + mancozeb 64%) 72% WP (76.73%), (tricyclazole 18% + mancozeb 62%) 80% WP (77.64%), (captan 70% + hexaconazole 5%) 75% WP (77.43%) and (azoxystrobin 11% + tebuconazole 18.3%) 29.3% SC (78.50%). In all the fungicides, inhibition of mycelial growth increased with increase in concentrations. The results obtained were similar to the results of Pondkule *et al*. (2020) [17] who reported that (azoxystrobin 18.2% +difenoconazole 11.4%) exhibited the highest percent inhibition (94.44%) at 1500 ppm. Azoxystrobin works by inhibiting the mitochondrial respiration in fungi, disrupting their energy production and ultimately leading to their death. While, difenoconazole works by inhibiting the biosynthesis of ergosterol, a crucial component of fungal cell membranes. Without ergosterol, the cell membranes become weakened and permeable, leading to cell death.

Table 1: *In vitro* evaluation of non-systemic fungicides against *Alternaria solani*

Sl. No.	fungicides	Percent inhibition of mycelial growth of fungus			
		Concentration (%)			Mean
		0.1	0.2	0.25	
1	Chlorothalonil 75% WP (Kavach 75%WP)**	55.00 (47.93)*	59.13 (50.27)	63.50 (52.95)	59.21 (50.38)
2	Mancozeb 75% WP (Indofil M-45 75% WP)	73.50 (59.12)	78.87 (62.80)	81.75 (64.73)	78.04 (62.06)
3	Propineb 70% WP (Antracol 70% WP)	68.00 (55.56)	68.63 (56.14)	76.63 (61.47)	71.08 (57.73)
4	Zineb 75% WP (Dithane Z-78 75% WP)	57.88 (49.62)	65.25 (54.02)	68.68 (56.13)	64.00 (53.26)
	Mean	63.59 (52.88)	67.97 (55.54)	72.64 (58.46)	
		S.Em. ±		C.D. at 1%	
	Fungicide (F)	0.57		1.62	
	Concentration (C)	0.49		1.41	
	F × C	0.99		3.58	

* Arc sine values
** Trade names

Table 2: *In vitro* evaluation of systemic fungicides against *Alternaria solani*.

Sl. No.	Fungicides	Percent inhibition of mycelial growth of fungus			
		Concentration (%)			Mean
		0.025	0.05	0.1	
1	Difenoconazole 25% EC (Score 25% EC)**	78.54 (62.47)*	82.50 (65.35)	85.63 (67.74)	82.22 (65.06)
2	Hexaconazole 5% EC (Contaf 5% EC)	77.08 (61.56)	81.25 (64.39)	82.50 (65.33)	80.28 (63.64)
3	Propiconazole 25% EC (Tilt 25% EC)	73.96 (59.38)	72.71 (58.80)	80.63 (63.89)	75.76 (60.51)
4	Tebuconazole 25.9% EC (Folicur 25.9% EC)	64.79 (53.62)	72.29 (58.25)	85.83 (67.98)	74.31 (59.55)
5	Thiophanate methyl 70% WP (Melvin 70% WP)	17.71 (24.66)	51.04 (45.58)	59.79 (50.65)	42.85 (40.30)
6	Validamycin 3% L	36.88 (37.36)	59.17 (50.48)	73.75 (59.50)	56.60 (48.79)
	Mean	58.16 (49.70)	69.83 (56.68)	78.02 (62.04)	
		S.Em. ±		C.D. at 1%	
Fungicide (F)		0.85		2.46	
Concentration (C)		0.60		1.74	
F × C		1.48		4.25	

* Arc sine values
** Trade names

Table 3: *In vitro* evaluation of combi product fungicides against *Alternaria solani*

Sl. No.	Fungicides	Percent inhibition of myceliail growth of fungus			
		Concentration (%)			Mean
		0.1	0.2	0.3	
1	(Zineb 68% + Hexaconazole 4%) 72% WP (Avtar 72% WP)**	66.04 (54.37)*	68.13 (55.64)	72.29 (58.25)	68.82 (56.09)
2	(Carbendazim 12% + Mancozeb 63%) 75% WP (Kapeni 75% WP)	67.29 (55.12)	68.13 (55.63)	72.92 (58.65)	69.44 (56.46)
3	(Azoxystrobin 18.2% + Difenoconazole 11.4%) 29.6% EC (Godiwa Super 29.6% EC)	73.96 (59.32)	78.13 (62.15)	84.17 (66.56)	78.75 (62.68)
4	(Flusilozole 12.5% + Carbendazim 25%) 37.5% EC (Lustre 37.5% EC)	64.79 (53.60)	77.29 (61.54)	78.96 (62.70)	73.68 (59.28)
5	(Metalaxyl 8% + Mancozeb 64%) 72% WP (Macto 72% WP)	70.42 (57.06)	78.96 (62.70)	80.83 (64.04)	76.73 (61.27)
6	(Tricyclazole 18% + Mancozeb 62%) 80% WP (Merger 80% WP)	72.50 (58.39)	79.17 (62.86)	81.25 (64.35)	77.64 (61.83)
7	(Tebuconazole 50% + Trifloxystrobin 25%) 75% WG (Nativo 75% WG)	68.33 (55.76)	73.75 (59.21)	82.71 (65.46)	74.93 (60.14)
8	(Captan 70% + Hexaconazole 5%) 75% WP (Taqat 75% WP)	69.17 (56.28)	80.00 (63.46)	83.13 (65.75)	77.43 (61.83)
9	(Azoxystrobin 11% + Tebuconazole 18.3%) 29.3% SC (Spectrum 29.3% SC)	70.08 (61.41)	81.67 (64.57)	83.75 (66.24)	78.50 (64.10)
10	(Hexaconazole 5% + Validamycin 2.5%) 7.5% SC (Valxtra 7.5% SC)	70.00 (56.79)	76.25 (60.84)	79.17 (62.87)	75.13 (60.16)
	Mean	69.25 (56.32)	76.15 (60.77)	79.86 (63.33)	
		S.Em. ±		C.D. at 1%	
Fungicide (F)		0.44		1.25	
Concentration (C)		0.24		0.68	
F × C		0.76		2.16	

* Arc sine values
** Trade names

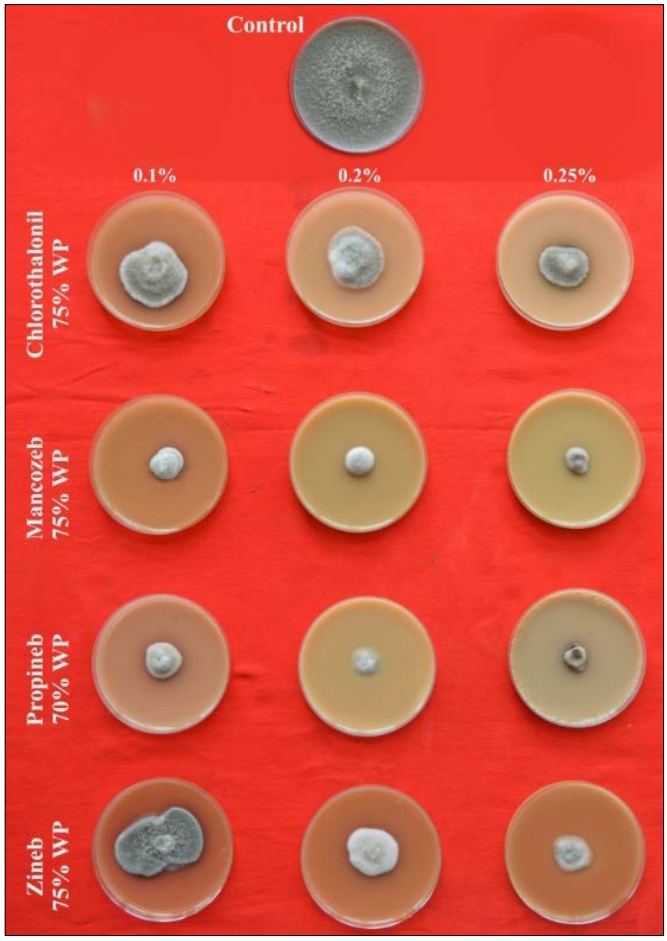

Plate 1: *in vitro* efficacy of non-systemic fungicides on inhibition of mycelial growth of *Alternaria solani*

Plate 2: *in vitro* efficacy of systemic fungicides on mycelial growth inhibition of *Alternaria solani*

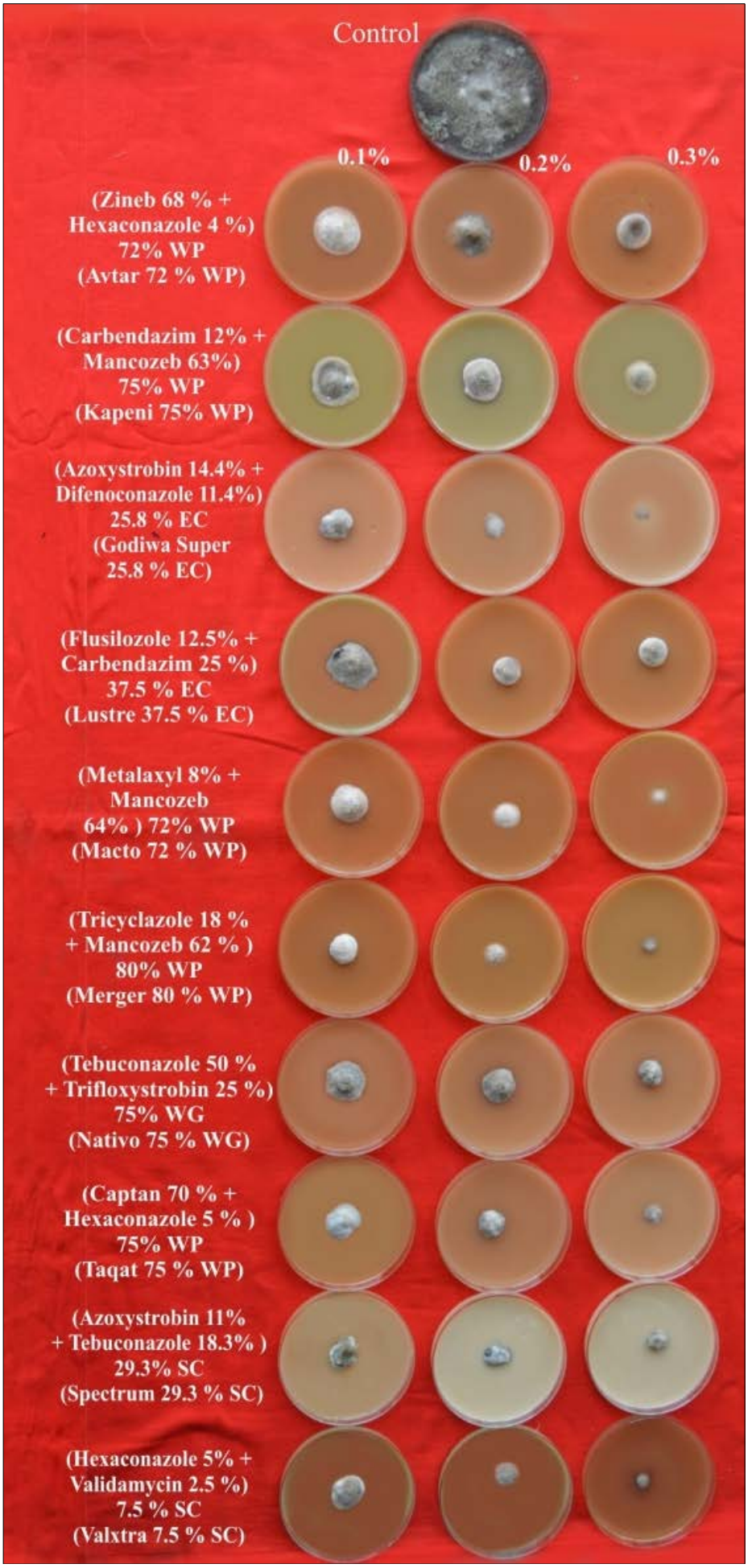

Plate 3: *In vitro* efficacy of combi product fungicides on inhibition of mycelial growth of *Alternaria solani*

Conclusion

Among non-systemic fungicides, irrespective of concentrations tested, mancozeb 75% WP recorded maximum mean percent mycelial inhibition (78.04%) followed by propineb 70% WP (71.08%) and least percent mycelial inhibition was recorded in chlorothalonil 75% WP (59.21%) Among systemic fungicides tested, difenoconazole recorded highest inhibition of mycelial growth (82.22%) and least mycelial growth inhibition was observed in thiophanate methyl (42.85%).

Among ten combi product fungicides, irrespective of fungicide concentrations (azoxystrobin 18.2% + difenoconazole 11.4%) 29.6% SC (78.75%) was found best in inhibiting mycelial growth of *Alternaria solani* and remain on par with (metalaxyl 8% + mancozeb 64%) 72% WP (76.73%), (tricyclazole 18% + mancozeb 62%) 80% WP (77.64%), (captan 70% + hexaconazole 5%) 75% WP (77.43%) and (azoxystrobin 11% + tebuconazole 18.3%) 29.3% SC (78.50%). In all the fungicides, inhibition of mycelial growth increased with increase in concentrations.

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