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Arati V Gajera
M. Sc. Student, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

KK Kanzaria
Associate Professor, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

Shruti G Solanki
M. Sc. Student, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

Kajal K Bariya
M. Sc. Student, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

Corresponding Author:
Arati V Gajera
M. Sc. Student, Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India

In vitro evaluation of fungitoxic potential of different fungicides against *Alternaria alternata* (Fr.) Keissler causing Alternaria leaf blight of isabgol

Arati V Gajera, KK Kanzaria, Shruti G Solanki and Kajal K Bariya

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Abstract
Isabgol (*Plantago ovata* Forsk.) is an important export-oriented medicinal crop of India, which dominates the global market for its seeds and husk. The crop is known to infect by many fungal diseases. Among them, Alternaria leaf blight caused by *Alternaria alternata* (Fr.) Keissler has become a serious threat for successful isabgol cultivation in Saurashtra. Considering the economic importance of disease, a laboratory experiment was conducted to study the efficacy of different non-systemic fungicides at 1000, 1500 and 2000 ppm; systemic fungicides at 100, 250 and 500 ppm and ready-mixed fungicides at 500, 750 and 1000 ppm concentrations against *A. alternata* by using Poisoned Food Technique. The results revealed that, among non-systemic fungicides, copper hydroxide 53.8 DF at 2000 and 1500 ppm concentration remained equally effective with mycelial growth inhibition of 92.82 and 92.46 per cent, respectively. In contrast, chlorothalonil 75 WP found less effective at 1000 ppm concentration (57.08%). Among systemic fungicides, propiconazole 25 EC and tebuconazole 25.9 EC each at 100, 250 and 500 ppm concentration gave cent per cent inhibition of mycelial growth of *A. alternata*. While, thiophanate methyl 70 WP found less effective at 100 ppm concentration (28.60%). Among ready-mixed fungicides, hexaconazole 4 + zineb 68 WP at 500, 750 and 1000 ppm concentration completely inhibited the mycelial growth of *A. alternata*. While, kresoxim methyl 15 + chlorothalonil 56 WG at 500 ppm concentration found least effective (67.84%) under *in vitro* condition.

Keywords: Isabgol, Alternaria leaf blight, *Alternaria alternata*, fungicides, mycelial growth inhibition

1. Introduction

Isabgol (*Plantago ovata* Forsk.) also known as blond psyllium in English and locally as "Ghodajiru" or "Uthmujiru" in Gujarat, is an important medicinal crop belonging to the family Plantaginaceae (2n = 8). It is widely cultivated in India, particularly in Gujarat and Rajasthan. The crop is best suited to well-drained, light sandy to sandy loam soils, which are considered most productive for its cultivation. It can tolerate slight salinity and grows well in soils with a pH range of 7.2 to 7.9. It is a short-duration crop and considered non-exhaustive due to its relatively low nutrient requirements (Das and Trivedi, 2023) [5].

The crop is primarily valued for its seed husk, commonly known as psyllium, which is extensively used in pharmaceutical, nutraceutical and food industries as a natural laxative due to its high mucilage content and therapeutic properties. The psyllium husk is rich in carbohydrates (84.98%) and also contains protein (0.94%) and ash (4.07%). Protein fractionation reveals the presence of albumin (35.8%), globulin (23.9%) and prolamin (11.7%) (Guo *et al*., 2008; Verma and Mogra, 2013) [8] [16]. India dominates the global production and export of isabgol contributing the majority share to the international market (Rahn, 1996) [12].

Despite its economic importance, isabgol productivity is significantly affected by several fungal diseases. Among them, Alternaria leaf blight caused by *Alternaria alternata* (Fr.) Keissler has emerged as a serious constraint, particularly in the Saurashtra region of Gujarat. The disease is characterized by small necrotic lesions on leaves that enlarge and coalesce, leading to blighting, reduced photosynthetic area and significant losses in seed yield and quality (Mandal, 2010) [10].

Effective management of Alternaria leaf blight is essential for sustaining isabgol production. Among various control measures strategies, fungicides are widely used due to their rapid and reliable action against fungal pathogens. Therefore, screening of fungicides under *in vitro* condition is a crucial step to evaluate their efficacy against *A. alternata*. Such studies provide baseline information for selecting suitable fungicides for field application to manage the disease. Hence, the present investigation was undertaken to assess the efficacy of different fungicides against Alternaria leaf blight of isabgol under laboratory condition.

2. Materials and Methods

The experiment was conducted in laboratory condition at Department of Plant Pathology, College of Agriculture, Junagadh Agricultural University, Junagadh during the year 2024-25. Seven different non-systemic, systemic and ready-mixed fungicides each with three different concentrations were tested against *A. alternata* using Poisoned Food Technique of Bagchi and Das (1968) [1]. An experiment was laid out in Completely Randomized Design (factorial concept) with three repetitions.

The experiment comprises with seven non-systemic fungicides *viz*., chlorothalonil 75 WP, copper hydroxide 53.8 DF, copper oxychloride 50 WP, mancozeb 75 WP, metiram 70 WG, propineb 70 WP and zineb 75 WP at 1000, 1500 and 2000 ppm concentrations; seven systemic fungicides *viz*., azoxystrobin 23 SC, carbendazim 50 WP, difenoconazole 25 EC, tebuconazole 25.9 EC, kresoxim methyl 44.3 SC, propiconazole 25 EC and thiophanate methyl 70 WP at 100, 250 and 500 ppm concentrations and seven ready-mixed fungicides *viz*., azoxystrobin 18.2 + difenoconazole 11.4 SC, metiram 55 + pyraclostrobin 5 WG, carbendazim 12 + mancozeb 63 WP, fluxapyroxad 25 + pyraclostrobin 25 SC, hexaconazole 4 + zineb 68 WP, kresoxim methyl 15 + chlorothalonil 56 WG and tebuconazole 50 + trifloxystrobin 25 WG at 500, 750 and 1000 ppm concentrations.

Appropriate quantity of each fungicide required was incorporated into autoclaved 100 ml PDA medium in 250 ml conical flask before solidification using digital balance and micropipette for solid and liquid formulation of fungicides, respectively. After thoroughly shaking on the vertex, the medium was poured into sterilized Petri plates (90 mm dia.) in equal quantity (20 ml per Petri plate) to form a uniform layer. The plates were then allowed to solidify. After solidification, an actively growing mycelial bit of *A. alternata* of 4 mm diameter cut with the help of sterilized cork-borer were transferred under aseptic conditions in an inverted position in the center of the plate to make a direct contact with solidified PDA medium. Then Petri plates were incubated at 28 ± 1 °C till the control plate attains full growth and observations were recorded on radial mycelial growth in treated and control plates. Inoculated Petri plates containing PDA medium without fungicides served as control (Grover and Moore, 1962) [7].

The per cent mycelial growth inhibition of the fungus in each of the treatment was calculated by using the following formula (Vincent, 1947) [17].

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

Where,

I = Per cent inhibition of mycelial growth

C = Mean radial growth of fungus in control plate (mm)

T = Mean radial growth of fungus in treated plate (mm)

2.1 Statistical analysis

Statistical analysis was carried out as per standard methods as suggested by Panse and Sukhatme (1985) [11]. Actual percentage data were converted to arcsine values and analyzed statistically.

3. Results and Discussion

The observations on mycelial growth inhibition exerted by non-systemic, systemic and ready-mixed fungicides were recorded after nine days of inoculation. The results obtained are communicated here under.

3.1 Efficacy of Non-Systemic Fungicides Against *A. alternata In vitro*

The data presented in Table 1, Figure 1 and Plate I-A revealed that all non-systemic fungicides were capable of reducing the mycelial growth of *A. alternata in vitro* as compared to control.

Among different non-systemic fungicides tried *in vitro*, the maximum mean mycelial growth inhibition of 91.24 per cent observed in copper hydroxide 53.8 DF. Whereas, the least mean mycelial growth inhibition of 59.55 per cent was found in chlorothalonil 75 WP.

All the three concentrations of non-systemic fungicides tested against *A. alternata* found effective in inhibiting the mycelial growth of *A. alternata* over control. As the concentration of fungicides increases, the mycelial growth inhibition of *A. alternata* also increases.

Among the three concentrations, 2000 ppm concentration remained significantly superior with mycelial growth inhibition of 80.67 per cent.

While, looking to the interaction effect between the non-systemic fungicides and various concentrations, copper hydroxide 53.8 DF at 2000 and 1500 ppm concentration found equally effective with mycelial growth inhibition of 92.82 and 92.46 per cent, respectively. The next effective treatment in order of merit was copper hydroxide 53.8 DF (88.45%) at 1000 ppm, but it was found at par with copper oxychloride 50 WP at 2000 and 1500 ppm concentration with mycelial growth inhibition of 88.05 and 86.93 per cent, respectively. While, chlorothalonil 75 WP found the least effective treatment as compared to other fungicides and had minimum mycelial growth inhibition of 57.08, but it was remained statistically at par with the same fungicide at 1500 ppm concentration with mycelial growth inhibition of 58.01 per cent.

A more or less similar type of results were also reported by Ladumor *et al*. (2019) [9]. They observed copper hydroxide 50 WP at 2500 ppm concentration with 85.51 per cent mycelial growth inhibition against *Alternaria cucumerina* causing leaf blight of bottle gourd and chlorothalonil 75 WP (37.89, 43.98, 50.63 and 52.00%) as the least effective even at 1000, 1500, 2000 and 2500 ppm concentration, respectively.

3.2 Efficacy of Systemic Fungicides Against *A. alternata In vitro*

The data presented in Table 2, Figure 2 and Plate I-B revealed that all systemic fungicides were capable of reducing the mycelial growth of *A. alternata in vitro* as compared to control.

Among different systemic fungicides tried, tebuconazole 25.9 EC and propiconazole 25 EC found significantly superior over rest of the treatments with cent per cent mean mycelial growth inhibition. The treatment with minimum order of merit with respect to mean mycelial growth inhibition was found in thiophanate methyl 70 WP (42.15%) followed by carbendazim 50 WP (44.66%).

All the three concentrations of systemic fungicides tested against *A. alternata* found effective in inhibiting the mycelial growth of *A. alternata* over control. As the concentration of fungicides increases, the mycelial growth inhibition of *A. alternata* also increases.

Among the three concentrations, 500 ppm concentration remained significantly superior over rest of the concentrations with 72.81 per cent mean mycelial growth inhibition.

The interaction effect between systemic fungicides and concentrations shows that, propiconazole 25 EC and tebuconazole 25.9 EC at all the three concentration remained equally effective and significantly superior over rest of the treatments with cent per cent mycelial growth inhibition of *A. alternata*. Difenoconazole 25 EC at 500 ppm concentration found next effective treatment in order of merit with 90.63 per cent mycelial growth inhibition, but it was found statistically at par with same fungicide at 250 ppm concentration with 88.77 per cent mycelial growth inhibition. Whereas, thiophanate methyl 70 WP found the least effective treatment as compared to other treatment and had minimum mycelial growth inhibition of 28.60 per cent at 100 ppm concentration.

The present findings were in close conformity with the results of Thaware *et al.* (2010) [15], Chauhan and Ravi (2020) [3], Bajaya *et al.* (2022) [2] and Shekhada *et al.* (2023) [14]. They reported propiconazole 25 EC as highly effective treatment with cent per cent mycelial growth inhibition of. *A. alternata* under *in vitro* condition.

3.3 Efficacy of Ready-Mixed Fungicides Against *A. alternata In vitro*

The data presented in Table 3, Figure 3 and Plate I-C revealed that all ready-mixed fungicides were capable of reducing the mycelial growth of *A. alternata in vitro* as compared to control.

Among all ready-mixed fungicides, hexaconazole 4 + zineb 68 WP found significantly superior over rest of the treatments with cent per cent mean mycelial growth inhibition of test pathogen. The next effective treatment in order of merit was tebuconazole 50 + trifloxystrobin 25 WG with 93.91 per cent mean mycelial growth inhibition, but it was remained statistically at par with azoxystrobin 18.2 + difenoconazole 11.4 SC with 93.06 per cent mean mycelial growth inhibition. The treatment with minimum mean mycelial growth inhibition was observed in kresoxim methyl 15 + chlorothalonil 56 WG (72.53%) followed by carbendazim 12 + mancozeb 63 WP (76.92%).

All the three concentrations of ready-mixed fungicides tested against *A. alternata* found effective in inhibiting the mycelial growth of *A. alternata* over control. As the concentration of fungicides increases, the mycelial growth inhibition of *A. alternata* also increases.

Among the three concentrations of ready-mixed fungicides, 1000 ppm concentration remained significantly superior over rest of the concentrations with 88.51 per cent mean mycelial growth inhibition.

The interaction effect between the ready-mixed fungicides and concentrations, hexaconazole 4 + zineb 68 WP at all the three concentration completely inhibited the mycelial growth of *A. alternata in vitro* and proved significantly superior over rest of the treatments. The treatment tebuconazole 50 + trifloxystrobin 25 WG found next effective treatment at 1000 ppm concentration and gave 94.29 per cent mycelial growth inhibition, but it was found at par with azoxystrobin 18.2 + difenoconazole 11.4 SC (94.11%) at the same concentration and tebuconazole 50 + trifloxystrobin 25 WG (93.18%) at 750 ppm concentration. While, kresoxim methyl 15 + chlorothalonil 56 WG at 500 ppm concentration remained the least effective as compared to other treatments and had minimum mycelial growth inhibition of 67.84 per cent.

More or less similar type of trend using ready-mixed fungicides were also reported by different scientists while working with *A. alternata*. Rokade *et al.* (2024) [13] concluded that tebuconazole 50 + trifloxystrobin 25 WG completely inhibited the mycelial growth of *A. alternata* of linseed at 500 ppm concentrations. The effectiveness of tebuconazole 50 + trifloxystrobin 25 WG and azoxystrobin 18.2 + difenoconazole 11.4 SC also reported by Chavda and Gohel (2022) [4] and Ginoya and Gohel (2015) [6] while working with *A. alternata* which are in line with the present findings.

Table 1: Efficacy of non-systemic fungicides against *A. alternata in vitro*

Non-systemic fungicides	Mycelial growth inhibition (%)*			Mean mycelial growth inhibition (%)
	1000 ppm	1500 ppm	2000 ppm	
Chlorothalonil 75 WP	49.07 (57.08)	49.61 (58.01)	52.88 (63.57)	50.52 (59.55)
Copper hydroxide 53.8 DF	70.13 (88.45)	74.06 (92.46)	74.46 (92.82)	72.88 (91.24)
Copper oxychloride 50 WP	66.23 (83.75)	68.81 (86.93)	69.78 (88.05)	68.27 (86.24)
Mancozeb 75 WP	62.19 (78.23)	62.95 (79.32)	64.30 (81.20)	63.15 (79.58)
Metiram 70 WG	56.02 (68.76)	62.16 (78.20)	67.26 (85.06)	61.81 (77.34)
Propineb 70 WP	61.92 (77.84)	63.87 (80.61)	64.96 (82.09)	63.59 (80.18)
Zineb 75 WP	55.56 (68.02)	57.40 (70.98)	57.99 (71.91)	56.99 (70.30)
Mean	60.16 (74.59)	62.69 (78.07)	64.52 (80.67)	-
	Fungicide (F)	**Concentration (C)**		**F x C**
S. Em. ±	0.41	0.27		0.71
C. D. at 5%	1.16	0.76		2.01
C.V.%	1.96			

Note: * Mean of three repetitions

Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

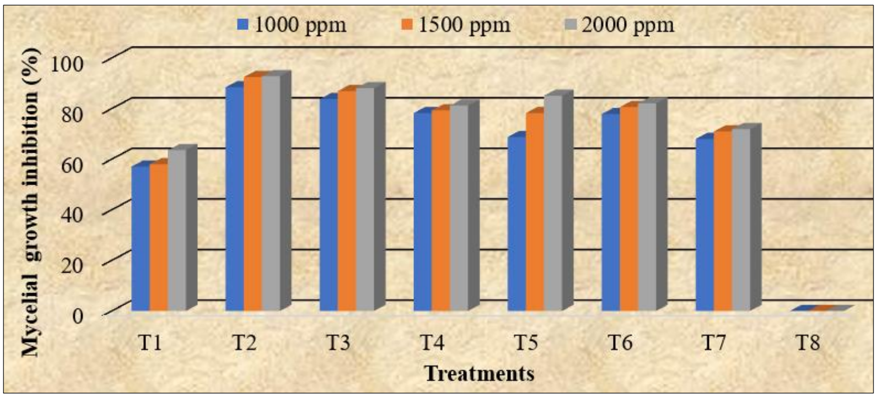


Fig 1: Efficacy of non-systemic fungicides against *A. alternata in vitro*

Table 2: Efficacy of systemic fungicides against *A. alternata in vitro*

Systemic fungicides	Mycelial growth inhibition (%)*			Mean mycelial growth inhibition (%)
	100 ppm	250 ppm	500 ppm	
Azoxystrobin 23 SC	44.07 (48.37)	46.19 (52.08)	46.72 (53.00)	45.66 (51.15)
Carbendazim 50 WP	35.39 (33.53)	42.68 (45.96)	47.58 (54.49)	41.88 (44.66)
Difenoconazole 25 EC	67.55 (85.42)	70.42 (88.77)	72.17 (90.63)	70.05 (88.27)
Tebuconazole 25.9 EC	90.05 (100.00)	90.05 (100.00)	90.05 (100.00)	90.05 (100.00)
Kresoxim methyl 44.3 SC	44.70 (49.48)	46.30 (52.26)	50.15 (58.94)	47.05 (53.56)
Propiconazole 25 EC	90.05 (100.00)	90.05 (100.00)	90.05 (100.00)	90.05 (100.00)
Thiophanate methyl 70 WP	32.33 (28.60)	42.25 (45.22)	46.51 (52.63)	40.36 (42.15)
Mean	57.73 (63.63)	61.13 (69.18)	63.32 (72.81)	-
	Fungicide (F)	**Concentration (C)**		**F x C**
S. Em. ±	0.42	0.28		0.73
C. D. at 5%	1.21	0.79		2.09
C.V.%	2.09			

Note: * Mean of four repetitions
Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

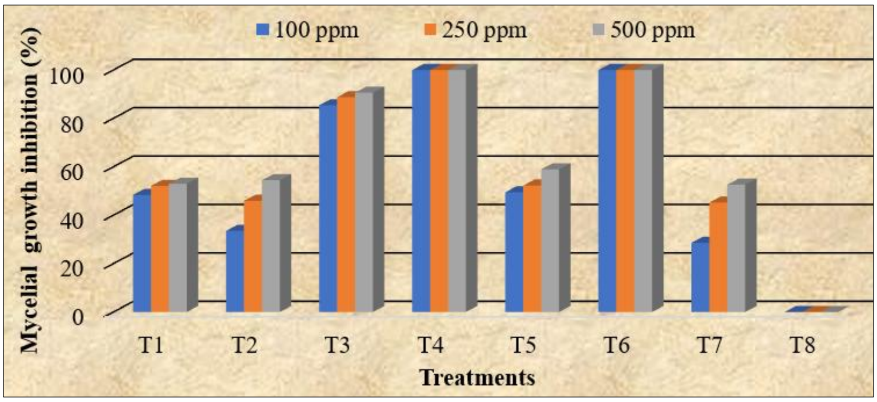


Fig 2: Efficacy of systemic fungicides against *A. alternata in vitro*

Table 3: Efficacy of ready-mixed fungicides against *A. alternata in vitro*

Ready-mixed fungicides	Mycelial growth inhibition (%)*			Mean mycelial growth inhibition (%)
	500 ppm	750 ppm	1000 ppm	
Azoxystrobin 18.2 + Difenoconazole 11.4 SC	73.65 (92.07)	74.66 (93.00)	75.95 (94.11)	74.75 (93.06)
Metiram 55 + Pyraclostrobin 5 WG	64.17 (81.01)	64.28 (81.17)	67.40 (85.23)	65.28 (82.47)
Carbendazim 12 + Mancozeb 63 WP	58.71 (73.02)	59.93 (74.90)	65.53 (82.84)	61.39 (76.92)
Fluxapyroxad 25 + Pyraclostrobin 25 SC	67.70 (85.61)	68.00 (85.97)	68.95 (87.09)	68.22 (86.22)
Hexaconazole 4 + Zineb 68 WP	90.05 (100.00)	90.05 (100.00)	90.05 (100.00)	90.05 (100.00)
Kresoxim methyl 15 + Chlorothalonil 56 WG	55.45 (67.84)	59.18 (73.76)	60.65 (75.98)	58.43 (72.53)
Tebuconazole 50 + Trifloxystrobin 25 WG	74.05 (92.44)	74.87 (93.18)	76.18 (94.29)	75.03 (93.31)
Mean	69.11 (84.57)	70.14 (86.00)	72.10 (88.51)	-
	Fungicide (F)	**Concentration (C)**		**F x C**
S. Em. ±	0.31	0.20		0.53
C. D. at 5%	0.87	0.57		1.51
C.V.%	1.30			

Note: * Mean of four repetitions
Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values.

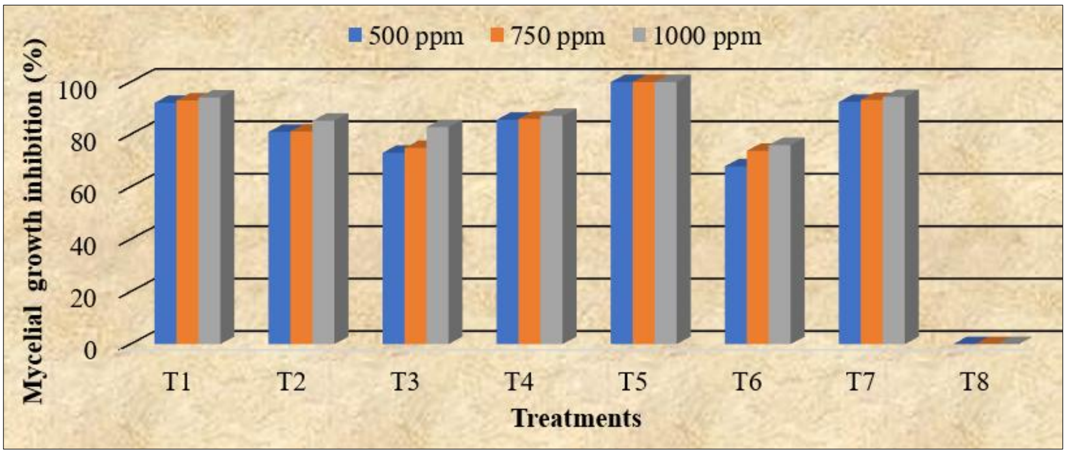
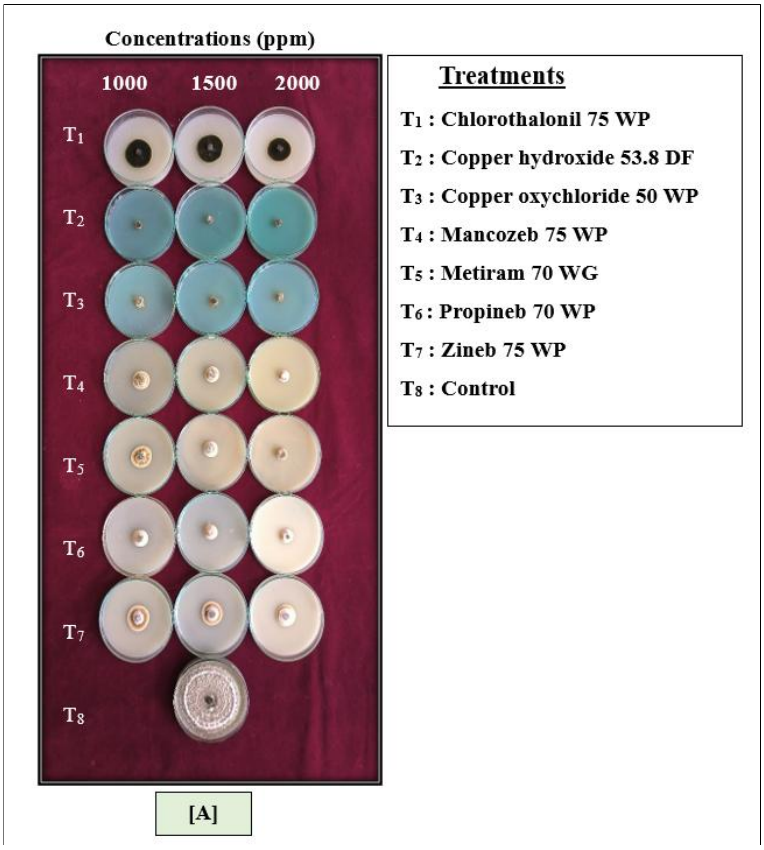


Fig 3: Efficacy of ready-mixed fungicides against *A. alternata in vitro*



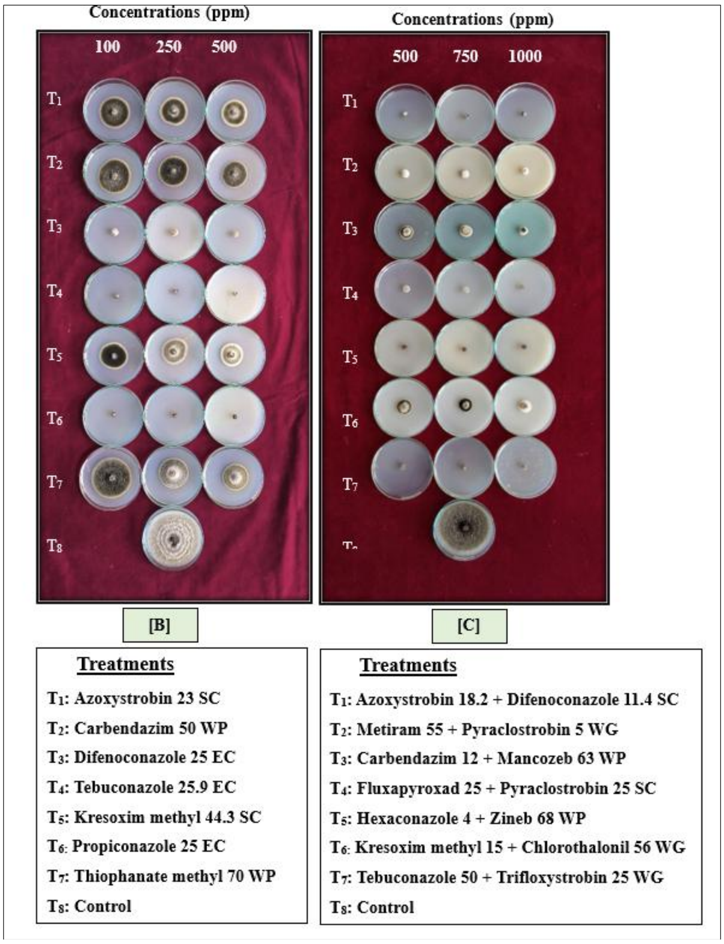


Plate I: Efficacy of non-systemic [A], systemic [B] and ready-mixed [C] fungicides against *A. alternata in vitro*

4. Conclusion

Based on the present investigation on "*In vitro* Evaluation of Fungitoxic Potential of different fungicides against *Alternaria alternata* (Fr.) Keissler causing Alternaria leaf blight of isabgol", it can be concluded that copper hydroxide 53.8 DF among non-systemic fungicides at 2000 and 1500 ppm concentration gave 92.82 and 92.46 per cent mycelial growth inhibition of *A. alternata*. Propiconazole 25 EC and tebuconazole 25.9 EC among systemic fungicides each at 100, 250 and 500 ppm concentration and hexaconazole 4 + zineb 68 WP among ready-mixed fungicide at 500, 750 and 1000 ppm concentration gave cent per cent mycelial growth inhibition of *A. alternata* under laboratory condition.

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