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# *In vitro* efficacy of fungicides against *Alternaria alternata* (Fr.) Keissler, causing Leaf Blight disease of Chrysanthemum

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**Abstract**
Chrysanthemum {*Dendranthema indicum* (= *Chrysanthemum indicum* L.)}, crop is being affected by several fungal, bacterial, viral and nematode induced diseases. However, leaf blight, caused by *Alternaria alternata* (Fr.) Keissler has been common occurrence, causing quantitative as well as qualitative losses in chrysanthemum. Therefore, present *in vitro* study was planned in CRD with two separate experiments (Contact and systemic and combi-fungicides) *in vitro*, in 13 treatments replicated thrice, to assess the efficacy of fungicides against *Alternaria alternata*, at the Department of Plant Pathology, College of Agriculture, Latur (MS), during *Rabi*, 2019-20. Results revealed that, all of the six systemic (each @ 500, 1000 and 1500 ppm), five contact and two combi-products fungicides (each @ 1000, 2000 and 2500 ppm) evaluated *in vitro* were found fungistatic and exhibited significant mycelial growth inhibition of *A. alternata*, causing *Alternaria* leaf blight of chrysanthemum. However, the systemic fungicides *viz*., Propiconazole 25% EC and Hexaconazole 5% EC (100%), followed by Difenconazole 25% EC (87.81%), Penconazole 10% EC (86.13%) were found most effective against the test pathogen. Whereas, among contact / combi – fungicides the most effective fungicide found were Carbendazim 12% WP + Mancozeb 63% WP with highest growth inhibition (81.96%), followed by Mancozeb 75% WP (78.05%), Copperoxy chloride 50% WP (69.36%), respectively.

**Keywords:** Chrysanthemum, *Alternaria alternata*, fungicides, inhibition

## Introduction

Chrysanthemum (*Chrysanthemum indicum* L.) occupies a key position in the floriculture industry and it's the world's second most important floricultural crop after Rose (Kalia, 2015). It is also called as 'Queen of East' in European countries and commonly called as gul-e-daudi or golden flower autumn queen. Chrysanthemums belong to the Asteraceae (Compositae) family. It is the best dollar earning flower in United States; also grown in India and Maharashtra as a cut flower. In India, chrysanthemum commercially growing major states are Karnataka, Tamilnadu, Maharashtra, Rajasthan, Madhya Pradesh and Bihar. In Maharashtra, chrysanthemum is grown on an area of 0.39 thousand ha with the production of 1.65 thousand tonnes loose flowers and 0.05 thousand tonnes cut flowers (Anonymous, 2018) [1]. In Maharashtra, the leading districts in floriculture production are Nasik, Ahmednagar, Thane, Pune, Satara, Sangli and Nagpur. However, Ahmednagar district is specalized as growing district of the Maharashtra (Tupe *et al*., 2017) [18].

Various biotic diseases are threatening the cultivation and good quality bloom yield of chrysanthemum. Among them, major disease are leaf blight (*Alternaria alternata*), leaf spot (*Septoria chrysanthemella*), wilts (*Fusarium* and *Verticillium* spp.), root rot (*Pythium* spp., *Phytophthora* spp.), powdery mildew (*Golovinomyces chrysanthemi*), dry root rot (*Rhizoctonia solani*), brown rust (*Puccinia chrysanthemi*), bacterial crown galls (*Agrobacterium tumefaciens*), bacterial blight (*Pseudomonas cichori*),viral stunt, mosaic and nematodes (Pradeepkumar *et al*., 2008) [14]. Among of these diseases, leaf blight caused by *Alternaria alternata* (Fries.) Keissler is one of the most destructive disease, commonly prevailing in almost all chrysanthemum growing areas and consequently causing accountable quantitative losses (> 80% yield losses) as well as deteriorating the quality of produce (Arunkumar, 2008; Divyajyothi *et al*., 2018) [3, 7].

Therefore, present study on *in vitro* efficacy of fungicides against *A. alternata*, causing chrysanthemum *Alternaria* leaf blight was planned and conducted at the Department of Plant Pathology, College of Agriculture, Latur, during *Rabi*, 2019-20.

## Materials and Methods

**Isolation, identification and pathogenicity of *A. alternata***

Applying tissue isolation technique (Tuite, 1969) [19], the fungus causing leaf blight disease in chrysanthemum was isolated from naturally infected chrysanthemum (Cv.White probiotic) specimen, on autoclaved and cooled potato dextrose agar (PDA) plates. Pathogenicity test attempted by spray inoculating the spore suspension ($2\times10^6$ spores / ml) of the test pathogen, on chrysanthemum (Cv.White probiotic) seedlings in pot culture.

Based on typical symptoms of *Alternaria* blight (naturally and artificially diseased chrysanthemum plants), pathogenicity test, morpho-cultural characteristics and microscopic observations, the test pathogen was identified as *Alternaria alternata* (Fries.) Keissler., the cause of chrysanthemum *Alternaria* blight and further confirmed by comparing its authentic descriptions (Ellis, 1971) [8].

***In vitro* evaluation of systemic fungicides:** Two separate experiments were planned to evaluate *in vitro* efficacy of six systemic (each @ 500, 1000 and 1500 ppm) and five non-systemic / contact plus two combi-products fungicides (each @ 1000, 2000 and 2500 ppm) and conducted with Completely Randomized Design (CRD) and all the treatments replicated thrice against *A. alternata*, causing leaf blight of chrysanthemum by applying Poisoned Food Technique (Nene and Thapliyal, 1993) [12] and using Potato Dextrose Agar as a basal culture medium. Observations on radial mycelial growth / colony diameter (mm) were in all the replicated treatments at 24 hrs interval and continued till growth of the test pathogen in untreated control plates was fully covered. Per cent inhibition of the test pathogen over untreated control was calculated by applying following formula (Vincent, 1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where, C = Growth of the test fungus in untreated control plates

T = Growth of the test fungus in treated plates

The data obtained was statistically analyzed (Panse and Sukhatme, 1978) [13] and the results were interpreted thereof.

## Result and Discussion

The results obtained on per cent mycelial growth inhibition of test pathogen A. *alternata*, with the test systemic fungicides are depicted in PLATE-I and presented in Table 1 and Fig. 1. The test non systemic and combi- product fungicides are depicted in PLATE-II and presented in Table 2 and Fig. 2.

**Mycelial growth inhibition of systemic fungicides:** Results (PLATE I, Table 1, Fig. 1) revealed that, the systemic fungicides tested (each @ 500, 1000 and 1500 ppm) significantly inhibited mycelial growth of *A. alternata*, over untreated control and it was directly proportional to concentrations of the test fungicides. Results (PLATE I, Table 1, Fig. 1) revealed average mycelial growth inhibition recorded with the systemic fungicides ranged from 35.35 to 100.00 per cent. However, it was highest with Propiconazole 25% EC and Hexaconazole 5% EC (100%), followed by Difenconazole 25% EC (87.81%), Penconazole 10% EC (86.13%), Carbendazim 50% WP (47.82%) and Thiophanate methyl 70% WP (35.35%), respectively, as against (0.00) mm with untreated control.

**Table 1:** *In vitro* efficacy of systemic fungicides against *A. alternata*, causing chrysanthemum blight

| Tr. No. | Treatments | Col. Dia.* (mm) at ppm | | | Av. (mm) | % Inhibition* at ppm | | | Av. Inhib. (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | 500 | 1000 | 1500 | | 500 | 1000 | 1500 | |
| $T_1$ | Propiconazole 25% EC | 00.00 (00.00) | 00.00 (00.00) | 00.00 (00.00) | 00.00 (00.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) |
| $T_2$ | Hexaconazole 05% EC | 00.00 (00.00) | 00.00 (00.00) | 00.00 (00.00) | 00.00 (00.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) | 100 (90.00) |
| $T_3$ | Difenconazole 25% EC | 13.34 (21.42) | 10.70 (19.09) | 8.86 (17.31) | 10.96 (19.33) | 85.17 (67.35) | 88.11 (69.82) | 90.15 (71.70) | 87.81 (69.56) |
| $T_4$ | Penconazole 10% EC | 13.59 (21.63) | 13.37 (21.44) | 10.48 (18.88) | 12.48 (20.68) | 84.90 (67.13) | 85.14 (67.32) | 88.35 (70.04) | 86.13 (68.13) |
| $T_5$ | Thiophanate methyl 70% WP | 65.20 (53.84) | 58.39 (49.82) | 50.92 (45.52) | 58.15 (49.69) | 27.55 (31.66) | 35.12 (36.34) | 43.40 (41.20) | 35.35 (36.48) |
| $T_6$ | Carbendazim 50% WP | 52.90 (46.66) | 47.52 (43.57) | 40.45 (39.49) | 46.95 (43.25) | 41.22 (39.94) | 47.20 (43.39) | 55.05 (47.89) | 47.82 (43.75) |
| $T_7$ | Control (untreated) | 90.00 (71.56) | 90.00 (71.56) | 90.00 (71.56) | 90.00 (71.56) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | **SE ±** | **0.44** | **0.49** | **0.48** | - | **0.51** | **0.38** | **0.44** | - |
| | **CD (P=0.01)** | **1.36** | **1.51** | **1.48** | - | **1.56** | **1.19** | **1.36** | - |

Thus, all of the six systemic fungicides tested were found anti-causal against *A. alternata* and significantly inhibited its mycelial growth. However, fungicides found most effective in their order of merit were, Propiconazole 25% EC and Hexaconazole 5% EC> Difenconazole 25% EC> Penconazole 10 EC> Carbendazim 50% WP and Thiophanate methyl 70% WP.

These results are in conformity to the reports of several earlier workers. Fungicides, Propiconazole 25% EC and Hexaconazole 5% EC were reported to cause maximum mycelial growth inhibition in many *Alternaria* spp. (Arunkumar and Kamanna, 2009; Apet *et al*., 2014; Thejakumar and Devappa 2016; Bhat *et al*., 2017; Shindhe *et* *al*., 2018; Vijayalakshmi *et al*., 2018; Mahadevaswamy *et al*., 2019).

**Mycelial growth inhibition of non-systemic and two combi-product fungicides:** All five non-systemic and two combi-product fungicides evaluated *in vitro* (each @ 1000, 2000 and 2500 ppm) were found effective in mycelia growth inhibition of *A. alternata* (PLATE-II, Table 2 and Fig. 2.) and it was increased with increasing concentrations of the test pathogen. Results (PLATE II, Table 2, Fig- 2) indicated that, non systemic and combi-product tested (each @ 1000, 2000 and 2500 ppm) significantly inhibited mycelial growth of *A. alternata* in the range of 48.50 to 78.60 per cent, 49.45 to 82.04 per cent and 58.08 to 85.25 per cent, respectively @1000, 2000 and 2500 ppm over untreated control and it was directly, proportional to the concentration of the fungicides tested. Average mycelial growth inhibition with the test fungicides ranged from 52.01 to 81.96 per cent. The fungicide, Carbendazim 12% WP + Mancozeb 63% WP showed highest growth inhibition (81.96%). It was followed by Mancozeb 75% WP (78.05%), Copperoxy chloride 50% WP (69.36%), Copper hydroxide 77% WP (67.22%), Propineb 70% WP (58.65%), Metalaxyl 8% + Mancozeb 64% WP (56.93%) and Chlorothalonil 75% WP (52.01%), respectively.

**Table 2:** *In vitro* efficacy of non systemic and combi-product fungicides

| Tr. No. | Treatments | Col. Dia.* (mm) at ppm | | | Av. (mm) | % Inhibition* at ppm | | | Av. Inhib. (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | 1000 | 2000 | 2500 | | 1000 | 2000 | 2500 | |
| $T_1$ | Chlorothalonil 75% WP | 46.35 (42.90) | 45.49 (42.41) | 37.72 (37.89) | 43.18 (41.08) | 48.50 (44.14) | 49.45 (44.68) | 58.08 (49.64) | 52.01 (46.15) |
| $T_2$ | Metalaxyl 8% + Mancozeb 64% WP | 40.27 (39.38) | 39.28 (38.80) | 36.72 (37.29) | 38.75 (38.49) | 55.25 (48.01) | 56.35 (48.64) | 59.20 (50.30) | 56.93 (48.98) |
| $T_3$ | Carbendazim 12 WP+ Mancozeb 63 WP | 19.26 (26.03) | 16.16 (23.70) | 13.27 (21.36) | 16.23 (23.75) | 78.60 (62.44) | 82.04 (64.92) | 85.25 (67.41) | 81.96 (64.86) |
| $T_4$ | Copperoxy chloride 50% WP | 31.46 (34.11) | 26.68 (31.09) | 24.57 (29.71) | 27.57 (31.67) | 65.04 (53.75) | 70.35 (57.00) | 72.70 (58.50) | 69.36 (56.39) |
| $T_5$ | Copper-hydroxide 77% WP | 32.96 (35.03) | 28.26 (32.11) | 27.27 (31.48) | 29.49 (32.89) | 63.37 (52.75) | 68.60 (55.91) | 69.70 (56.60) | 67.22 (55.07) |
| $T_6$ | Mancozeb 75% WP | 21.24 (27.44) | 20.50 (26.92) | 17.50 (24.72) | 19.74 (26.37) | 76.40 (60.93) | 77.22 (61.49) | 80.55 (63.83) | 78.05 (62.06) |
| $T_7$ | Propineb 70% WP | 40.14 (39.31) | 38.43 (38.31) | 33.07 (35.10) | 37.21 (37.58) | 55.40 (48.10) | 57.30 (49.19) | 63.25 (52.68) | 58.65 (49.98) |
| $T_8$ | Control (untreated) | 90.00 (71.56) | 90.00 (71.56) | 90.00 (71.56) | 90.00 (71.56) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | **SE ±** | **0.55** | **0.73** | **0.59** | **-** | **0.55** | **0.55** | **0.62** | **-** |
| | **CD (P=0.01)** | **1.67** | **2.21** | **1.80** | **-** | **1.66** | **1.67** | **1.89** | **-** |

* Mean of three replication

Figures in parentheses are arcsine transformed values

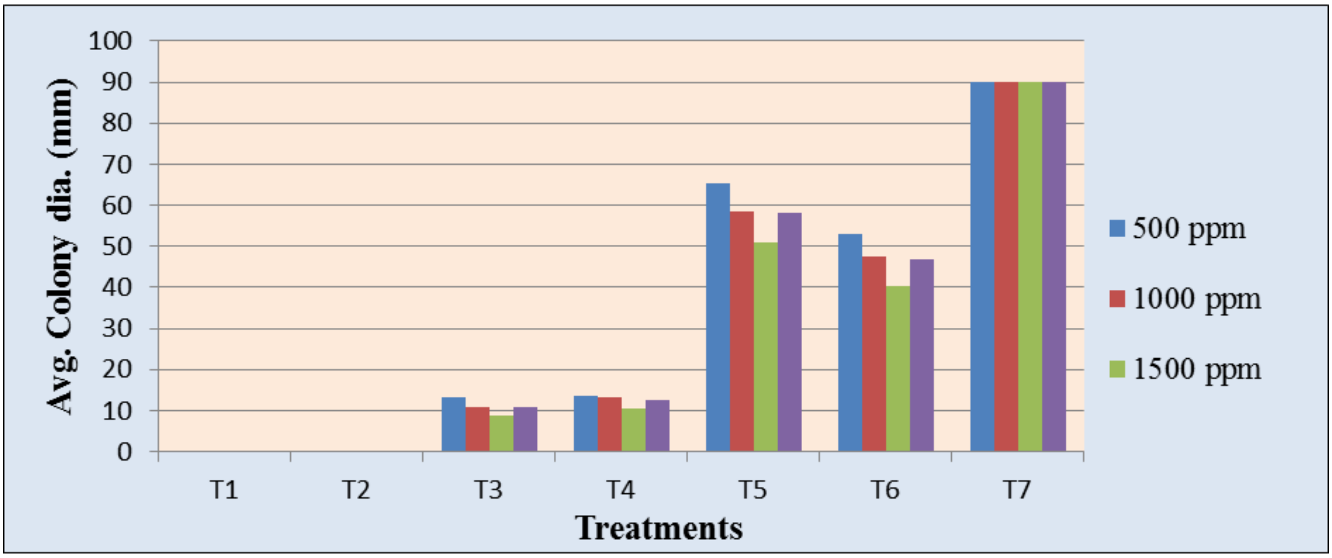
Thus, based on efficiency, fungicides in the order of merit were Carbendazim 12% + Mancozeb 63% WP> Mancozeb 75% WP> Copperoxy chloride 50% WP> Copper hydroxide 77% WP> Propineb 70% WP> Metalaxyl 8% + Mancozeb 64% WP and Chlorothalonil 75% WP, respectively.

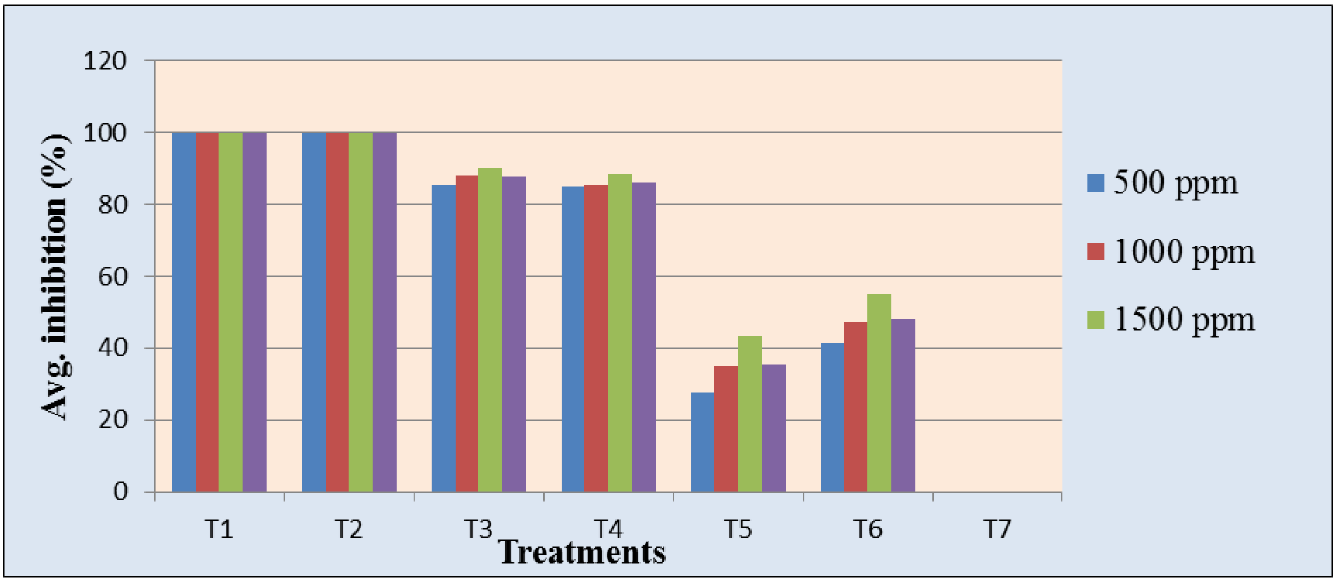
These results of the present study are in consonance with the findings of many earlier workers, who reported effectiveness of non-systemic fungicides such as Propineb 70% WP, Mancozeb75% WP, Copperoxy chloride 50% WP, Copper hydroxide 77% WP, Chlorothalonil 75% WP etc. against many phytopathogenic *Alternaria* spp. (Bavaji *et al*., 2012; [5] Apet *et al*., 2014; Thejakumar and Devappa, 2016; Bhat *et al*., 2017; Shindhe *et al*., 2018; Vijayalakshmi *et al*., 2018; Mahadevaswamy *et al*., 2019), combi-product such as Carbendazim 12% + Mancozeb 63% WP, Metalaxyl 8%+ Mancozeb 64% WP against many phytopathogenic *Alternaria* spp. (Gholve *et al*., 2014 [9]; Shamala and Janardhana, 2015) [9, 15].

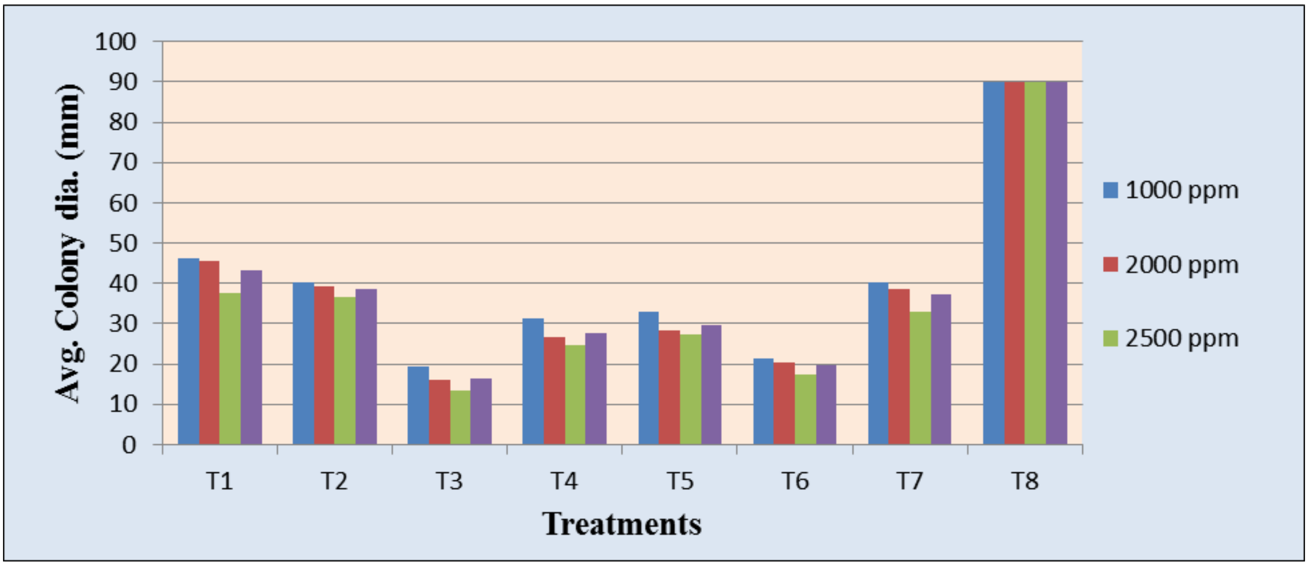

**Plate I:** *In vitro* efficacy of systemic fungicides against *A. alternata*, causing chrysanthemum blight


1000 ppm 2000 ppm 2500 ppm

**Plate II:** *In vitro* efficacy of non- systemic / combi-product fungicides against *A. alternata*, causing chrysanthemum blight





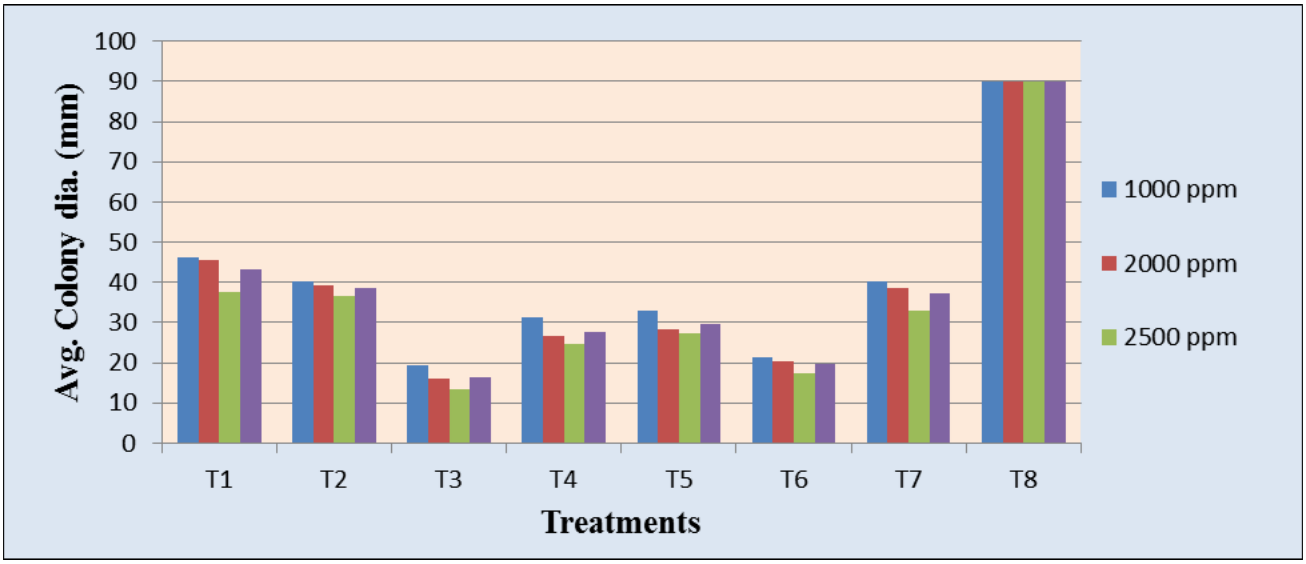




**Fig 1:** *In vitro* efficacy of systemic fungicides against *A. alternata*, causing chrysanthemum leaf blight

## Conclusion

Hence, from ongoing results and discussion, it is concluded that, all of the seven systemic (each @500, 1000 and 1500 ppm) and five contact and two combi-product fungicides (@1000, 2000 and 2500 ppm) evaluated *in vitro* were found effective and fungistatic against *A. alternata*. However, Based on average mycelial growth inhibition, the systemic fungicides *viz*., Propiconazole 25% EC, Hexaconazole 5% EC, Difenconazole 25% EC, Penconazole 10 EC, Carbendazim 50% WP and Thiophanate methyl 70% WP were found most effective against the test pathogen. Whereas, among contact / combi-product fungicides, the most effective fungicides found were Carbendazim 12% + Mancozeb 63% WP, Mancozeb 75% WP, Copperoxy chloride 50% WP, Copper hydroxide 77% WP, Propineb 70% WP, Metalaxyl 8% + Mancozeb 64% WP and Chlorothalonil 75% WP, respectively.

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