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# *In vitro* effect of different phyto-extracts and fungicides against *Colletotrichum graminicola* (Ces.) Wilson

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**Abstract**
Sorghum belongs to family Poaceae. It is the fifth most important world cereal crop after wheat, rice, maize and barley. Anthracnose caused by *Colletotrichum graminicola* (Ces.) Wilson is one of the most destructive foliar diseases in sorghum. Considering this, the present investigation on effect of different phyto-extracts and systemic, non-systemic and combined fungicides at different concentrations were tested by poisoned food technique *in vitro* to know their inhibitory effect on the growth of anthracnose pathogen. Garlic clove extract at 10 and 20 per cent concentration showed cent per cent growth inhibition of *C. graminicola*. Among the systemic fungicides, the cent per cent growth inhibition of the fungus was recorded in carbendazim at 100, 250, 500 and 1000 ppm concentrations and propiconazole at 500 and 1000 ppm concentrations. Among the non-systemic fungicides, the cent per cent growth inhibition of the fungus was recorded in copper oxychloride at 1500, 2000 and 2500 ppm and mancozeb at 2500 ppm concentrations. Among the combined fungicides, the cent per cent growth inhibition of the fungus was recorded in carbendazim 12 % + mancozeb 63 % at 500, 1000 1500 and 2000 ppm concentrations.

**Keywords:** Sorghum, anthracnose, *colletotrichum graminicola*, phyto-extracts, fungicides and poisoned food

## 1. Introduction
Sorghum is one of the world's leading cereal crops. Sorghum is used for food, fodder and the production of alcoholic beverages (Reddy *et al.*, 2006) [9]. Sorghum is cultivated over 42 million ha in the semi-arid regions of Asia, Americas, Australia and Africa. Asia alone contributes 30 per cent of world sorghum production. Sorghum production in Asia is concentrated mainly in India and China, which together contribute 86 per cent of Asia's total sorghum production. Sorghum is grown in areas receiving 500 to 1000 mm annual rainfall with the temperature requirement of 26 °C to 32 °C. (Rao *et al.*, 2004) [7]. In India prevalent foliar diseases of sorghum are rust, downy mildew, anthracnose, zonate leaf spot, leaf blight, grey leaf spot, sooty stripe and tar spot (Sharma *et al.*, 1978) [10]. The grain loss caused by anthracnose disease varies from region to region. It has been reported to be 1.2 to 16.4 per cent in India (Mishra and Siradhana, 1978) [5]. Anthracnose initially produced small, red, purple or brown spots with whitish or purple centers. The spots are elliptical or spindle shaped 2 to 4 mm long and 1 to 2 mm broad, surrounded by well-defined margin. The affected young seedling shows blighting. Infection is localized, fungus produce acervuli (Rangaswami and Mahadevan, 2010) [6]. In the present investigation, fungicides and phyto-extracts both have showed inhibitory effect against *Colletotrichum graminicola*. The main purpose of use of phyto-extracts was to reduce the residual effect of chemical on the plant and ecofriendly management of disease.

## 2. Materials and Methods
### 2.1 Isolation of pathogen
Sorghum plants showing characteristics symptoms of anthracnose were collected from farmer's field. The infected portion of leaf and stem was cut into small bits (2 to 3 mm) in such a way that each bit consist of infected and healthy tissues. These bits were transferred under aseptic condition in sterilized Petri plates containing 20 ml previously sterilized Potato Dextrose Agar (PDA) solidified medium. Petri plates were incubated for 3 days at 28 ºC ± 2 ºC temperature in an incubator for the fungus growth and obtain pure culture of pathogen.

### 2.2 Effect of different phyto-extracts against *Colletotrichum graminicola in vitro*

The effects of phyto-extracts of different plants were evaluated against *C. graminicola* under *in vitro* conditions by poisoned food technique. The fresh plant materials were collected and washed thoroughly with tap water and then finally with repeated changes of sterilised distilled water. The fresh plant materials were separately grinded in sterilised distilled water at the rate of one ml/g of the plant parts in a sterilised pestle and mortar. The extracts were filtered through two layers of muslin cloth and subsequently filtered through filter paper. This formed the standard plant extract solution (100 %). The extracts were centrifuged at the rate of 6000 RPM at 4°C for 10 minutes. All the plant extracts were used at 10 and 20 per cent concentrations. For 10 per cent concentration 10 ml of the plant extract was added to 90 ml of the sterilised warm PDA medium. For 20 per cent concentration 20 ml of the plant extract was added to 80 ml of the sterilised warm PDA medium. Then the medium were poured into the sterilised Petri plates under aseptic conditions. A five mm disc of seven days old culture of the pathogen were cut by means of a sterilized cork borer and placed at the center of the Petri plate. The plates were incubated at 28°C ± 2°C. The medium without incorporating the plant extract was served as control. The mycelial growth of the pathogen was measured when the control treatment with pathogen reached full growth. Three plates were maintained for each treatment. The per cent inhibition of mycelial growth was calculated by using following formula (Vincent, 1947) [13].

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

Where,
PGI = Per cent growth inhibition,
C = Average mycelial growth in control (mm), and
T = Average mycelial growth in treatment (mm).

### 2.3 Effect of different fungicides against *Colletotrichum graminicola in vitro*

The effect of fungicides on mycelial growth of *C. graminicola* was tested by poisoned food technique. Each fungicide was tested at four different concentrations. The required quantity of each test fungicide was added in conical flask containing 100 ml molten PDA medium so as to get required concentration in ppm. The flask containing poisoned medium was well shaken to facilitate uniform mixture of fungicide and 20 ml was poured in sterilised Petri plates. On solidification of the medium, the plates were inoculated with five mm disc of mycelial bit taken from the periphery of seven days old culture with the help of cork borer. The inoculated Petri plates were incubated at 28 °C ± 2 °C. Three Petri plates were used for each treatment. Petri plates without fungicide were served as control. The experiment was conducted in Completely Randomized Design (CRD). Colony diameter was measured along the two diagonals passing through the colony by excluding the initial diameter (5 mm) of bit. Colony diameter was measured when the control treatment with pathogen reached full growth. Per cent growth inhibition of fungus was calculated by using formula as mentioned in 2.2.

## 3. Results and Discussion

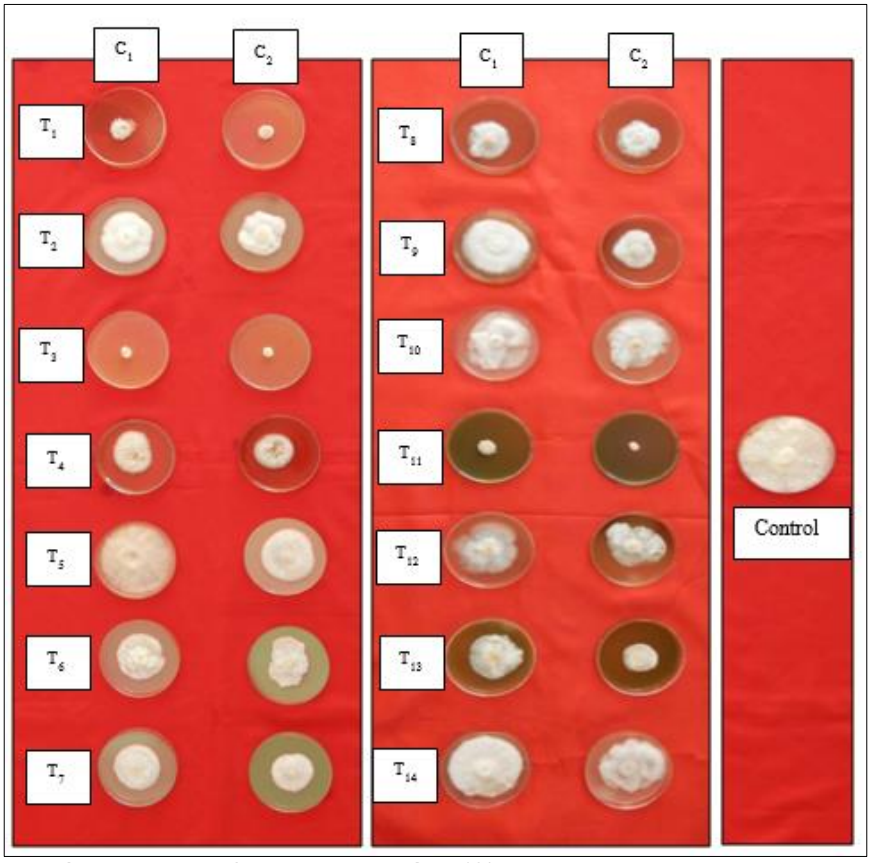
### 3.1 Effect of different phyto-extracts against *Colletotrichum graminicola in vitro*

In the present investigation, fourteen phyto-extracts were tested at 10 and 20 per cent concentrations with suitable control by poisoned food technique *in vitro* to know their inhibitory effect on the growth of *Colletotrichum graminicola*. The results presented in Table 1 revealed that all the plant extracts at 10 and 20 per cent concentration inhibited the growth of the pathogen significantly as compared to control. Garlic clove (*Allium sativum* L.) extract at 10 and 20 per cent concentrations showed cent per cent growth inhibition of *C. graminicola*. The next effective phyto-extracts at 20 per cent concentration, in order of inhibition were parthenium leaf (*Parthenium hysterophorus* L.) (100 %), onion bulb (*Allium cepa* L.) (82.75 %), gadar leaf (*Xanthium stremonium* L.) (58.24 %), ginger rhizome (*Zingiber officinale* Rosc.) (46.08 %) extracts. Piludi leaves (*Salvadora persica* L.) (14.12 % and 21.37 %), marigold leaves (*Tagetes erecta* L.) (3.92 % and 24.51 %) and nafatia leaves (*Ipomea fistulosa* L.) (21.18 and 26.08 %) extracts were found least effective in inhibiting the mycelial growth of *C. graminicola* at 10 and 20 per cent concentrations (Plate I). These results supported the observations of Singh *et al*. (1997) [12] and Alam *et al*. (2004) [2] reported that the extract of garlic was most effective in inhibiting mycelial growth of *Colletotrichum* pathogen in different crops. Kumar and Yadav (2007) [4] also reported that extract of *Allium sativum* at 4 per cent completely inhibited the growth of *Colletotrichum capsici*. The inhibitory property of garlic was due to the presence of antifungal chemical diallyl disulfide and diallyl thiosulfide and in parthenium due to presence of allelopathic compound parthenin.

**Table 1:** Effect of different phyto-extracts on growth inhibition of *C. graminicola in vitro*

| Sr. No. | Name of plants | Growth in hibition (%) / Concentration (%) | |
|---|---|---|---|
| | | 10 | 20 |
| 1 | *Allium cepa* L (Onion) | 69.61 (56.84)$^{d}$ | 82.75 (65.82)$^{c}$ |
| 2 | Datura | 32.75 (35.19)$^{j}$ | 36.47 (37.43)$^{i}$ |
| 3 | *Allium sativum* L. (Garlic) | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ |
| 4 | *Zingiber officinale* Rosc (Ginger) | 43.92 (41.78)$^{g}$ | 46.08 (43.01)$^{f}$ |
| 5 | *Tagetes erecta* L (Marigold) | 3.92 (11.91)$^{p}$ | 24.51 (29.98)$^{lm}$ |
| 6 | Neem | 34.90 (36.50)$^{i}$ | 41.57 (40.41)$^{h}$ |
| 7 | Tulsi | 29.80 (33.38)$^{k}$ | 35.69 (36.96)$^{i}$ |
| 8 | Barmasi | 43.14 (41.32)$^{gh}$ | 47.45 (43.80)$^{f}$ |
| 9 | Saragvo | 22.75 (28.80)$^{mn}$ | 42.94 (41.21)$^{gh}$ |
| 10 | Lantana | 23.53 (29.34)$^{m}$ | 29.41 (33.14)$^{k}$ |
| 11 | *Parthenium hysterophorus* L (Parthenium) | 80.20 (63.91)$^{c}$ | 100.00 (90.00)$^{a}$ |
| 12 | *Ipomea fistulosa* L.(Nafatia) | 21.18 (27.73)$^{n}$ | 26.08 (31.00)$^{l}$ |
| 13 | *Xanthium stremonium* L (Gadar) | 35.88 (37.08)$^{i}$ | 58.24 (50.01)$^{e}$ |
| 14 | *Salvadora persica* L. ( Piludi) | 14.12 (22.46)$^{o}$ | 21.37 (27.87)$^{n}$ |
| Plant × Concentration | | 0.62 | |
| S.Em. ± | | | |
| C.V. % | | 2.49 | |

Figures in parenthesis are arc sine transformed values; Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance.




T1- Onion
T2- Datura
T3- Garlic
T4- Ginger
T5- Marigold
T6- Neem
T7- Tulsi
T8- Barmasi
T9- Saragvo
T10- Lantana
T11- Parthenium
T12- Nafatia
T13- Gadar
T14- Piludi
C1- 10%
C2- 20%

**Plate I:** Effect of different phyto-extracts against *C. graminicolain vitro*

### 3.2 Effect of different fungicides against *Colletotrichum graminicola* in vitro

Five systemic, four non-systemic and four combined fungicides at different concentrations were tested *in vitro* for their comparative efficacy against *C. graminicola* through poisoned food technique. The results presented in Table 2 revealed that all the five systemic fungicides at different concentrations (100, 250, 500 and 1000 ppm) were found inhibitory to fungal growth. The cent per cent growth inhibition of the fungus was recorded in carbendazim at all the concentrations (100, 250, 500 and 1000 ppm) and propiconazole at 500 and 1000 ppm concentrations. The next best systemic fungicide was hexaconazole at 1000 ppm inhibited 87.84 per cent mycelial growth. Tebuconazole and difenconazole at 1000 ppm concentration inhibited 84.51 and 79.02 per cent fungal growth. Significantly least fungal growth inhibition was recorded in difenconazole (69.22 %) and tebuconazole (71.76 %) at 100 ppm concentration. The inhibitory effect of all the systemic fungicides increased with the increasing concentrations of the fungicides (Plate II).

**Table 2:** Effect of systemic fungicides on growth inhibition of *C. graminicola in vitro*

| Sr. No. | Fungicides | Growth inhibition(%) /Concentrations (ppm) | | | |
|---|---|---|---|---|---|
| | | 100 | 250 | 500 | 1000 |
| 1 | Difenconazole25 EC | 69.22 (56.60)$^{h}$ | 73.14 (59.08)$^{fg}$ | 76.86 (61.57)$^{e}$ | 79.02 (63.08)$^{d}$ |
| 2 | Hexaconazole 5 EC | 73.33 (59.21)$^{fg}$ | 78.63 (62.80)$^{d}$ | 79.02 (63.07)$^{d}$ | 87.84 (70.01)$^{b}$ |
| 3 | Propiconazole 25 EC | 85.29 (67.90)$^{c}$ | 88.24 (70.36)$^{b}$ | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ |
| 4 | Carbendazim 50 WP | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ |
| 5 | Tebuconazole 25.9 EC | 71.96 (58.32)$^{g}$ | 74.71 (60.11)$^{f}$ | 77.45 (61.98)$^{de}$ | 84.51 (67.20)$^{c}$ |
| Fungicide × Concentration | | 0.49 | | | |
| S.Em.± | | | | | |
| C.V. % | | 1.21 | | | |

-Figures in parenthesis are arc sine transformed values;
-Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance.

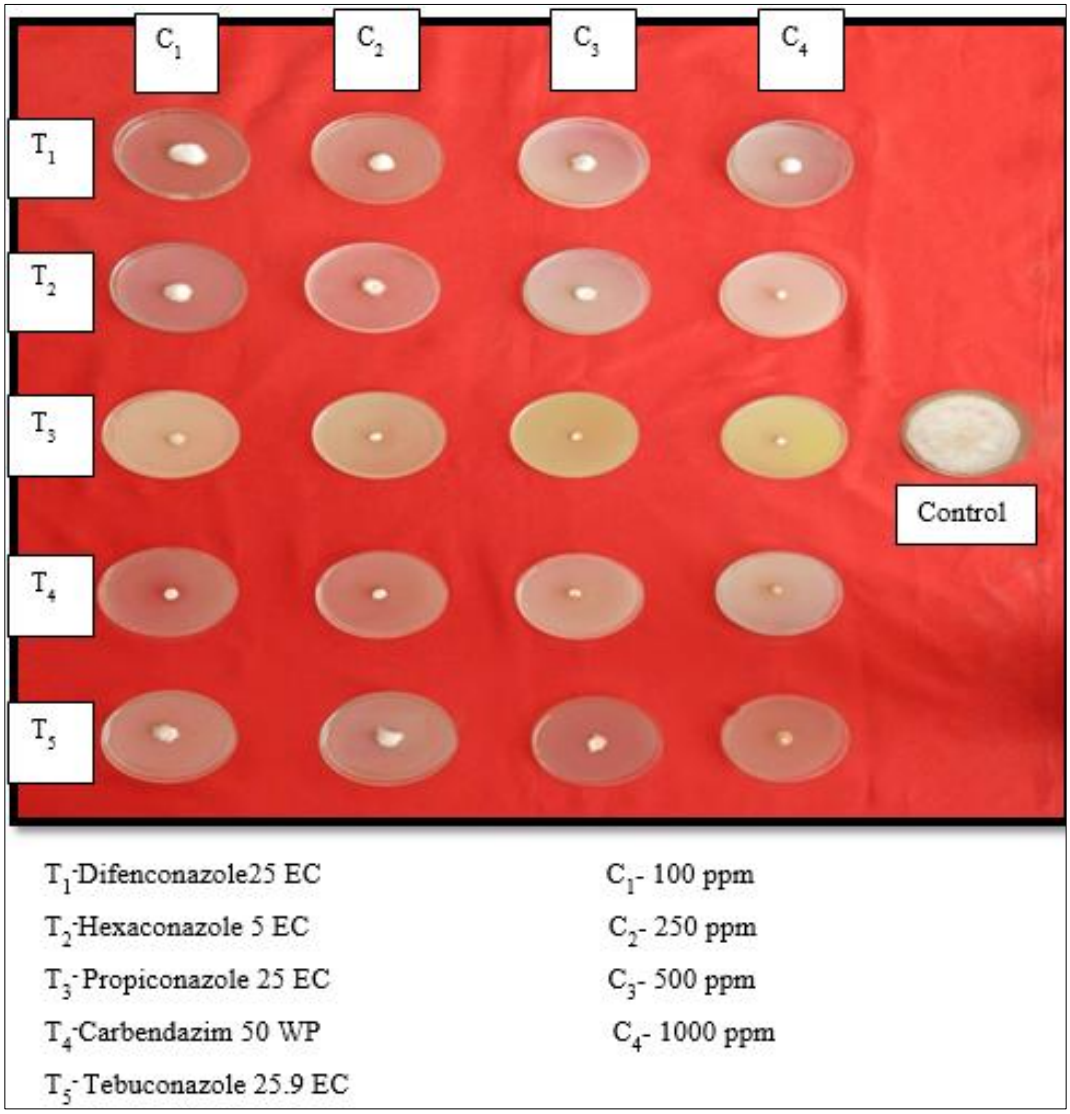



**Plate II:** Effect of systemic fungicides against *C. graminicola in vitro*

The results presented in Table 3 revealed that the non-systemic fungicides at different concentrations (1000, 1500, 2000 and 2500 ppm) were found inhibitory to the fungal growth. The cent per cent growth inhibition of the fungus was recorded in copper oxychloride at 1500, 2000 and 2500 ppm and mancozeb at 2500 ppm concentrations. The next best fungicide was propineb and chlorothalonil at 2500 ppm inhibited 84.12 and 76.86 per cent mycelial growth. The least effective fungicides were propineb and chlorothalonil at 1000 ppm concentration which inhibited 65.69 and 68.43 per cent fungal growth. Inhibitory effect of all the non-systemic fungicides increased positively with the increasing concentrations of the fungicides (Plate III).

**Table 3:** Effect of non-systemic fungicides on growth inhibition of C. graminicola in vitro

| Sr. No. | Fungicides | Growth inhibition(%) /Concentrations (ppm) | | | |
|---|---|---|---|---|---|
| | | 1000 | 1500 | 2000 | 2500 |
| 1 | Mancozeb 75 WP | 72.94 (58.97)$^{f}$ | 77.65 (62.11)$^{d}$ | 87.45 (69.66)$^{b}$ | 100.00 (90.00)$^{a}$ |
| 2 | Chlorothalonil 75 WP | 68.43 (56.10)$^{g}$ | 70.00 (57.09)$^{g}$ | 72.16 (58.45)$^{f}$ | 76.86 (61.57)$^{de}$ |
| 3 | Copper oxychloride 50 WP | 87.45 (69.67)$^{b}$ | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ |
| 4 | Propineb 70 WP | 65.69 (54.42)$^{h}$ | 75.29 (60.50)$^{e}$ | 76.27 (61.18)$^{de}$ | 84.12 (66.89)$^{c}$ |
| Fungicide × Concentration | | 0.51 | | | |
| S.Em.± | | | | | |
| C.V. % | | 1.31 | | | |

-Figures in parenthesis are arc sine transformed values;
-Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance.

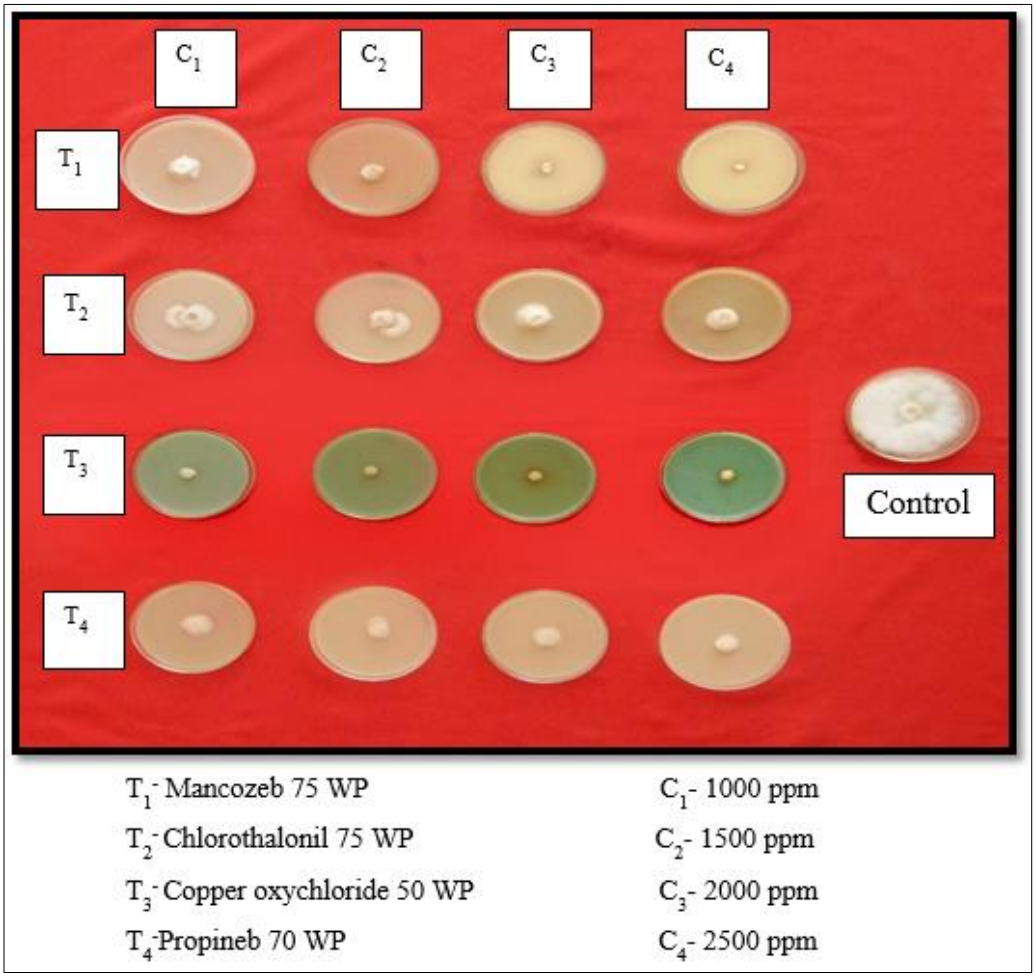



**Plate III:** Effect of non-systemic fungicides against *C. graminicolain vitro*

The results presented in Table 4 revealed that all the four combined fungicides at different concentrations (500, 1000, 1500 and 2000 ppm) were found inhibitory to fungal growth. The cent per cent growth inhibition of the fungus was recorded in carbendazim 12 % + mancozeb 63 % at all the concentrations. The next best combined fungicide was azoxystrobin 18.2 % + difenconazole 11.4 % inhibited 88.24 per cent mycelial growth followed by metalaxyl MZ 8 %+ mancozeb 64 % (87.06 %) and hexaconazole 4 % + Zineb 68 % (85.49 %) at 2000 ppm concentration. Least fungal growth inhibition was recorded in metalaxyl MZ 8 % + mancozeb 64 % at 500 ppm concentration (81.18 %). The inhibitory effect of all the combined fungicides increased with the increasing concentrations of the fungicides (Plate IV).

**Table 4:** Effect of combined fungicides on growth inhibition of C. graminicola in vitro

| Sr. No. | Fungicides | Growth inhibition(%)/Concentrations (ppm) | | | |
|---|---|---|---|---|---|
| | | 500 | 1000 | 1500 | 2000 |
| 1 | Carbendazim 12 % + Mancozeb 63 % WP | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ | 100.00 (90.00)$^{a}$ |
| 2 | Metalaxyl MZ 8 % + Mancozeb 64 % WP | 81.18 (64.63)$^{i}$ | 84.31 (67.06)$^{efg}$ | 85.29 (67.84)$^{de}$ | 87.06 (69.33)$^{bc}$ |
| 3 | Hexaconazole 4 % + Zineb 68 % WP | 82.94 (65.97)$^{gh}$ | 84.12 (66.88)$^{efg}$ | 84.90 (67.51)$^{def}$ | 85.49 (68.01)$^{cde}$ |
| 4 | Azoxystrobin 18.2% + Difenconazole 11.4 % SC | 81.57 (64.92)$^{hi}$ | 83.53 (66.42)$^{fg}$ | 86.27 (68.68)$^{cd}$ | 88.24 (70.36)$^{b}$ |
| Fungicide × Concentration | | 0.46 | | | |
| S.Em.± | | | | | |
| C.V. % | | 1.11 | | | |

-Figures in parenthesis are arc sine transformed values;
-Treatment means with the letter(s) in common are not significant by Duncan's New Multiple Range test at 5 per cent level of significance.

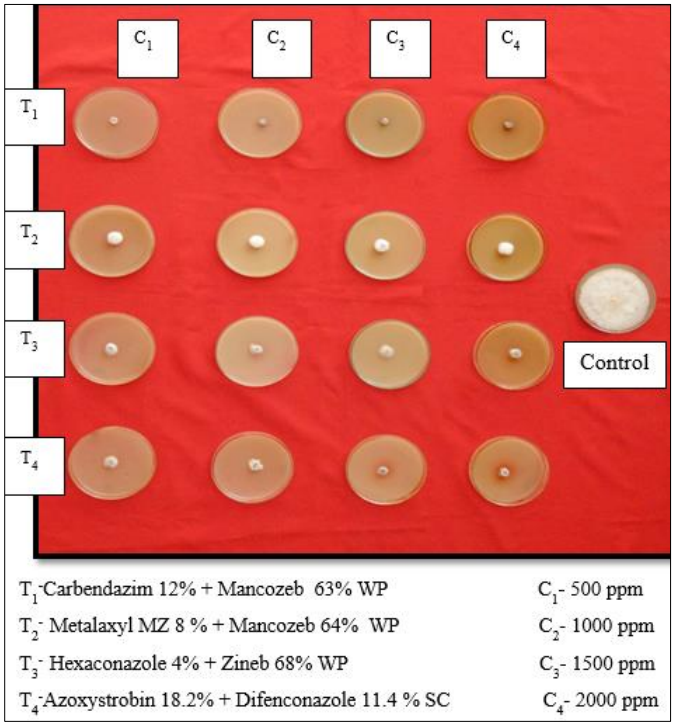



**Plate IV:** Effect of combined fungicides against *C. graminicola in vitro*

The present investigation was similar to the research work carried out by Akpa and Tsammani (1993) [1] who reported that in non-systemic fungicide mancozeb had the greatest inhibitory effect. Singh *et al*. (1999) [11] found that carbendazim was most effective in checking the mycelial growth of *C. graminicola*. Chaudhari and Gohel (2016) [3] reported that propiconazole, carbendazim 12 % + mancozeb 63 % completely inhibited the mycelial growth of *C. gloeosporioides*. Rewale *et al*. (2016) [8] reported that systemic fungicides propiconazole and difenconazole and in non-systemic fungicide mancozeb and copper oxychloride were found most effective in inhibiting the mycelial growth of *C. graminicola*.

## 4. References


1. Akpa AD, Tsammani DR. Effect of four fungicides against anthracnose of sorghum. Samaru Journal of Agricultural Research. 1993; 10:77-84.
2. Alam S, Han K, Lee J. *In vitro* effects of plant extract and phytohormons on mycelial growth of anthracnose fungi. The Korean Society of Mycology. 2004; 32(3):134-138.
3. Chaudhari KA, Gohel NM. Management of anthracnose disease of mungbean through new fungicidal formulations. Journal of Pure and Applied Microbiology. 2016; 10(1):691-696.
4. Kumar S, Yadav BP. Efficacy of Fungicides and Phyto-extract on *Colletotrichum* spp. Journal of Mycology and Plant Pathology. 2007; 37(2):336-364.
5. Mishra A, Siradhana BS. Chemical control of anthracnose of sorghum. Indian Phytopathology. 1978; 31:225-227.
6. Rangaswami G, Mahadevan A. Diseases of cereals. Diseases of Crop Plants in India. 4th Ed. Prentice-Hall of India Publication, 2010, 223-224.
7. Rao BD, Rana BS, Jyothi S, Hyma L, Karthikeyan K, Kumar KAB, *et al*. Importance and Economics of Sorghum and Pearl millet Production in Asia in alternative uses of sorghum and pearl millet in Asia, proceedings of the expert meeting ICRISAT, Patancheru, Andhra Pradesh, India 1st to 4th July (2003). CFC Technical Paper No. 2004; 34:14-41.
8. Rewale KA, Deshmukh RW, Gaikwad SH. *In vitro* evaluation of fungicides against *Colletotrichum graminicola* causing anthracnose of sorghum. International Journal of Tropical Agriculture. 2016; 34(6):1695-1700.
9. Reddy AR, Gowda C, Reddy B, Rai KN, Waliyar F, Alur AS *et al*. Enhanced Utilization Of Sorghum And Pearl Millet Grains In Poultry Feeds. An Indian Perspective. Department of Poultry Science, College of Veterinary Science, Rajendranagar, Hyderabad, India International Crops Research Institute for Semi-Arid Tropics, Patancheru, Hyderabad, India, 2006.
10. Sharma HC, Puranik KK, Jadhav MR, Jain NK. Sorghum diseases in Madhya Pradesh. Jawaharlal Nehru Krishi Viswa Vidyalaya Research Journal. 1978; 10:913.
11. Singh D, Chandra JP, Singh AB. Response of fungicides and antibiotics against anthracnose of sorghum caused by *Colletotrichum graminicola*. Indian Forester. 1999; 125:566-572.
12. Singh SN, Yadav BP, Sinha SK, Ojha KL. Efficacy of plant extracts in inhibition of radial growth and spore germination of *Colletotrichum capsici*. Journal of Applied Biology. 1997; 7:58-61.
13. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947; 159:850.