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# Assessing the *in vitro* efficacy of new molecules of fungicides against *Bipolaris setariae* infecting browntop millet

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## Abstract
*In vitro* efficacy of seventeen new molecules of fungicides were evaluated against *Bipolaris setariae* causing leaf blight on browntop millet at University of Agricultural Sciences, GKVK, Bangalore in order to find out effective fungicide against *B. setariae.* Mancozeb, among the six contact fungicides recorded cent per cent inhibition over control at 500 and 1000 ppm with mean inhibition of 96.54 per cent. Among the six systemic fungicides tested at 50, 100 and 150 ppm, propiconazole exerted 100 per cent inhibition of mycelial growth followed by tebuconazole which accounted 100 per cent inhibition at 100 and 150 ppm with mean inhibition of 94.69 per cent and cymoxanil + mancozeb among the five combi-product fungicides tested (100, 250 and 500 ppm) exhibited maximum (79.01 %) mean inhibition of mycelial growth. Among all groups, tricyclazole was found to be least effective.

**Keywords:** Browntop millet, *B. setariae*, Leaf blight, *In vitro* Fungicides

## Introduction
Millets are hardy crops that are adapted for cultivation in a range tropical and sub-tropical climate. Compared to cereals like rice and wheat which are consumed in large quantity over periods, millets are nutritionally superior and also less expensive besides having additionally high protein, vitamin and fibre content. As they are gluten free and having low glycemic index, millets serve as excellent food for diabetic and obese people. They are not only smart food but also smart crop by having photo insensitivity, climate resilience and drought tolerance ability.
Browntop millet (*Brachiaria ramosa* (L.) Stapf) is native to India (Oelke *et al.,* 1990) [1] and it was recently adopted into millets system in India as one of small millet for serving both food and fodder purpose. It is different from other small millets by having characters like shortest growth period, shade tolerant and suppressing root knot nematode population. In India, it is majorly cultivated in dry tracts of Andhra Pradesh-Karnataka border areas, Tamil Nadu and Maharashtra (Sujata *et al.*, 2018) [2]. Comparatively, millets production and productivity is lesser than the cereals and were further hindering their yield potentiality due to the biotic and abiotic stress. Majorly, biotic stress leads greater reduction in yield capability in which diseases occupies major part. Browntop millet leaf spot / leaf blight is caused by *B. setariae*.
Severity of browntop millet leaf blight was observed to be high in all the millet growing regions and was found to be one of the emerging diseases in India. However, no basic work was carried previously on various Integrated Disease Management (IDM) aspects of the pathogen. Hence, the present investigation was employed to identify suitable effective fungicides under *in vitro* conditions which is one of the pre-requisites for designing IDM approaches and evaluation under field conditions to mitigate disease under field conditions, there by the production and productivity of crop will be enhanced.

## Material and Methods
A total of six systemic, six combination product and five contact fungicides were tested in this study against *B. setariae* infecting browntop millet at different concentrations of 50, 100 and 150 ppm for systemic fungicides and 100, 250 and 500 ppm for combi-product fungicides while 250, 500 and 1000 ppm for contact fungicides on potato dextrose agar medium using poisoned food technique (Nene and Thapliyal, 1973 [3]; Sharvelle, 1961 [4]). Different fungicides evaluated were listed in table 1.
Sterilized potato dextrose agar was prepared and autoclaved. The medium was cooled to 40 °C. Fungicides were dissolved in sterilized water to make the stock solution. Appropriate quantity of stock solution was added to PDA to get the desired concentration of the fungicide;

the flasks were agitated gently to aid in uniform dispersion of the fungicidal solution into the medium. About 15 to 20 ml of poisoned PDA was poured into 90 mm Petri plates and allowed to solidify. One 6 mm disc of the actively growing culture of 9 days old fungus was transferred aseptically to centre of each Petri plates containing the poisoned medium. Control was maintained with the pathogen under similar conditions on PDA without poisoning the medium. Inoculated plates were incubated at 27±1 °C for 10 days and the colony diameter was recorded by measuring the radial growth of the fungus in three directions and the average diameter was calculated. Each treatment was replicated thrice. The per cent inhibition of the growth over control was determined (Vincent, 1947) [5].

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

Where,
I = Per cent inhibition. of mycelium
C= Growth of mycelium in control
T = Growth of mycelium in treatment

**Table 1:** List of fungicides used for *in vitro* evaluation against *B. setariae* infecting browntop millet with chemical and tradenames

| **Systemic fungicides** | | | | |
|---|---|---|---|---|
| **Sl. No.** | **Common name** | **Trade name & Concentration** | **Chemical name** | **Fungicide group** |
| 1 | Carbendazim | Bavistin (50 % WP) | Methyl 1H benzimidazol-2-yl carbamate | Benzimidazoles |
| 2 | Propiconazole | Tilt (25 % EC) | 1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole | Triazoles |
| 3 | Tricyclazole | Sivic (75 % WP) | 5-methyl-1, 2, 4-triazolo [3,4-b] [1,3] benzothiazole | |
| 4 | Tebuconazole | Folicur (25 % EC) | (RS)1 (4Chlorophenyl) 4,4 dimethyl-3 (1H, 1, 2, 4-triazol1ylmethyl) pentan3ol | |
| 5 | Thiophanate methyl | Roko (70 % WP) | Dimethyl 4, 4′-(*o*-phenylene) bis(3-thioallophanate) | Thiourea |
| 6 | Azoxystrobin | Amistar (23 % SC) | Methyl (2E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate | Strobilurins |
| **Combi-product fungicides** | | | | |
| **Sl. No.** | **Trade name** | **Concentration** | **Chemical name** | |
| 1 | Nativo | 75 % WG | Tebuconazole 50 % WG + Trifloxystrobin 25 % WG | |
| 2 | Custodia | 29.3 % SC | Azoxystrobin 11 % + Tebuconazole 18.3 % SC | |
| 3 | Curzate | 72 % WP | Cymoxanil 8 % + Mancozeb 64 % WP | |
| 4 | Merger | 80 % WP | Tricyclazole 18 % + Mancozeb 62 % WP | |
| 5 | Saaf | 75 % WP | Mancozeb 63 % + Carbendazim 12 % WP | |
| 6 | Amistar top | 32.5 % SC | Azoxystrobin 20 % + Difenoconazole 12.5 % SC | |
| **Contact (Non systemic) fungicides** | | | | |
| **Sl. No.** | **Common name** | **Trade name & Concentration** | **Chemical name** | **Fungicide group** |
| 1 | Propineb | Antracol (70 %WP) | Zinc proyllene-bis-dithocarbmate (polymeric) | Dithiocarbamate |
| 2 | Chlorothalonil | Kavach (75 % WP) | 2, 4, 5, 6-Tetrachloroisophthalonitrile | Organic compound |
| 3 | Mancozeb | Indofil M-45 (75 % WP) | Manganese ethylene bis (dithiocarbamate) (polymeric) complex with zinc salt | Dithiocarbamate |
| 4 | Zineb | Indofil Z-78 (75 % WP) | Zinc ethane-1, 2-di yl bis (dithiocarbamate) | Ethylene bisdithiocarbamte (EBDC) |
| 5 | Captan | Captan (50 % WP) | (3a*R*,7a*S*)-2-[(Trichloromethyl)sulfanyl]-3a, 4, 7, 7a-tetrahydro-1*H*-isoindole-1, 3(2*H*)-dione | Phthalimide |

### Statistical analysis
Experimental data was analysed in two factorial analysis using OPSTAT software developed by CCS HAU, Hisar. Also, angular transformation of the wide range values was done using WASP software developed by ICAR- Central Coastal Agricultural Research Institute, Goa. Inferences were drawn using 1 % level of significance for laboratory experiments.

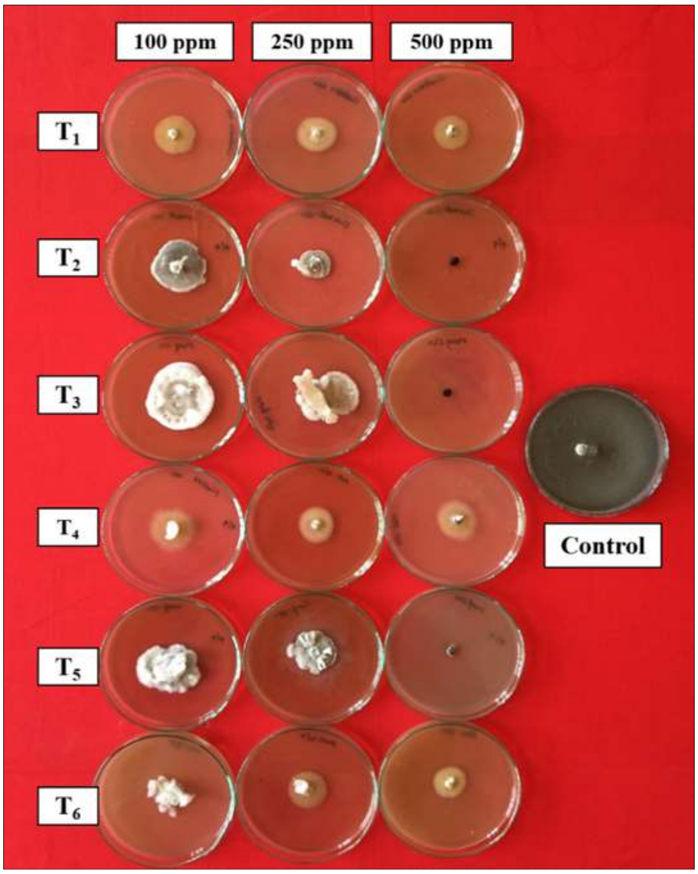
## Result and Discussion
### Efficacy of contact fungicides
Five contact fungicides namely captan, chlorothalonil, zineb, propineb and mancozeb were evaluated at three different (250, 500 and 1000 ppm) concentrations to test their efficacy against *B. setariae*. Among contact fungicides, mancozeb (96.54 %) exhibited maximum significant inhibition of mean mycelial growth whereas, other fungicides showed inhibition that ranged 76.91 - 82.50 %. Lowest % inhibition of mycelial growth was observed in zineb (76.91 %). With respect to different concentrations, 1000 ppm (73.95 %) recorded maximum percent growth inhibition while least observed in 250 ppm (64.50 %) (Table 2, Fig. 1 and Plate 1).

**Table 2:** *In vitro* efficacy of contact fungicides against *B. setariae* infecting browntop millet

| Sl. No. | Fungicide | Per cent inhibition over control* | | | Mean | Microscopic observation |
|---|---|---|---|---|---|---|
| | Concentration | 250 ppm | 500 ppm | 1000 ppm | | |
| 1 | Captan | 75.43 (60.28) | 80.49 (63.79) | 85.18 (67.36) | 80.37 (63.81) | Sporulation (+) |
| 2 | Chlorothalonil | 74.57 (59.71) | 77.90 (61.96) | 86.91 (68.79) | 79.79 (63.49) | Sporulation (-) |
| 3 | Zineb | 69.50 (56.48) | 76.29 (60.86) | 84.94 (67.16) | 76.91 (61.50) | Sporulation (-) |
| 4 | Propineb | 77.89 (61.95) | 82.96 (65.62) | 86.66 (68.58) | 82.50 (65.39) | Sporulation (-) |
| 5 | Mancozeb | 89.63 (71.21) | 100.00 (89.71) | 100.00 (89.71) | 96.54 (83.55) | Slime growth |
| | Mean | 64.50 (51.65) | 69.60 (57.04) | 73.95 (60.31) | 83.22 | |
| | | Fungicide (F) | Concentration (C) | | F × C | |
| | S.Em ± | 0.06 | 0.09 | | 0.15 | |
| | CD (P 0.01) | 0.26 | 0.18 | | 0.44 | |

**Note**: * Mean of three replications; -: No sporulation; +: 1-15 conidia per microscopic field; Figures in parenthesis are angular transformed values.

Of the fungicide and concentration interactions, absolute inhibition of mycelial growth was observed in mancozeb at 500 ppm and 1000 ppm. Other interactions showed moderate inhibition of 74.57 - 86.91 % at different concentrations. The lowest (69.50 %) mycelial growth inhibition was recorded in zineb at 250 ppm concentration.




**Treatment Details**
$T_1$- Captan 50 % WP
$T_2$- Chlorothalonil 75 % WP
$T_3$- Zineb 75 % WP
$T_4$- Propineb 70 % WP
$T_5$- Mancozeb 75 % WP

**Plate 1:** *In vitro* efficacy of different contact fungicides against *B. setariae*

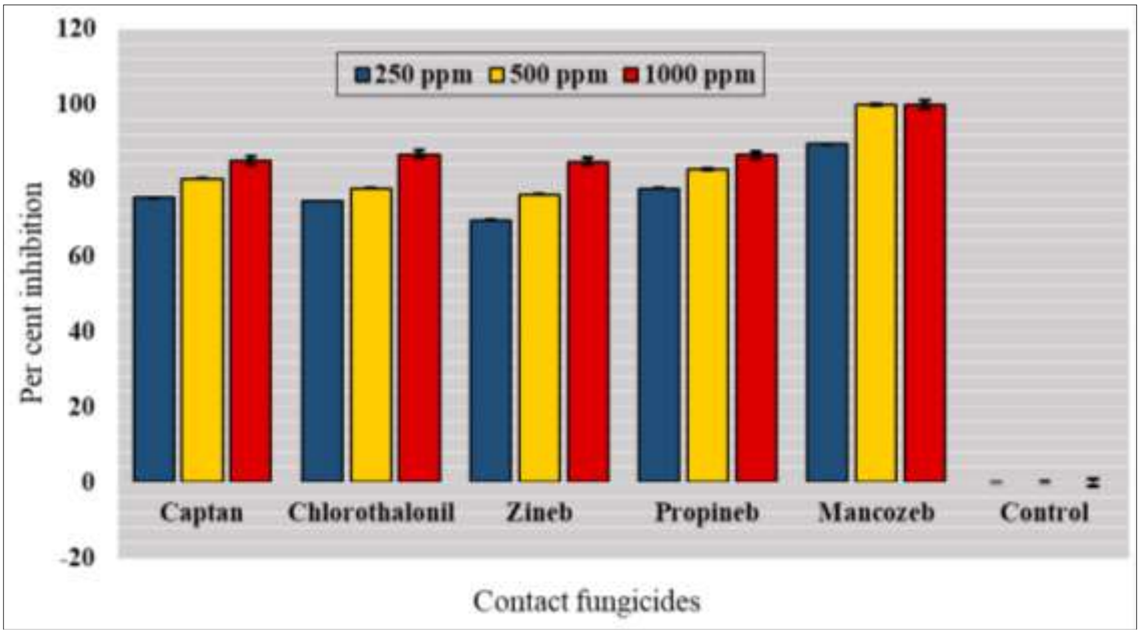



**Fig 1:** *In vitro* efficacy of contact fungicides on mycelial growth inhibition of *B. setariae*

**Efficacy of systemic fungicides**

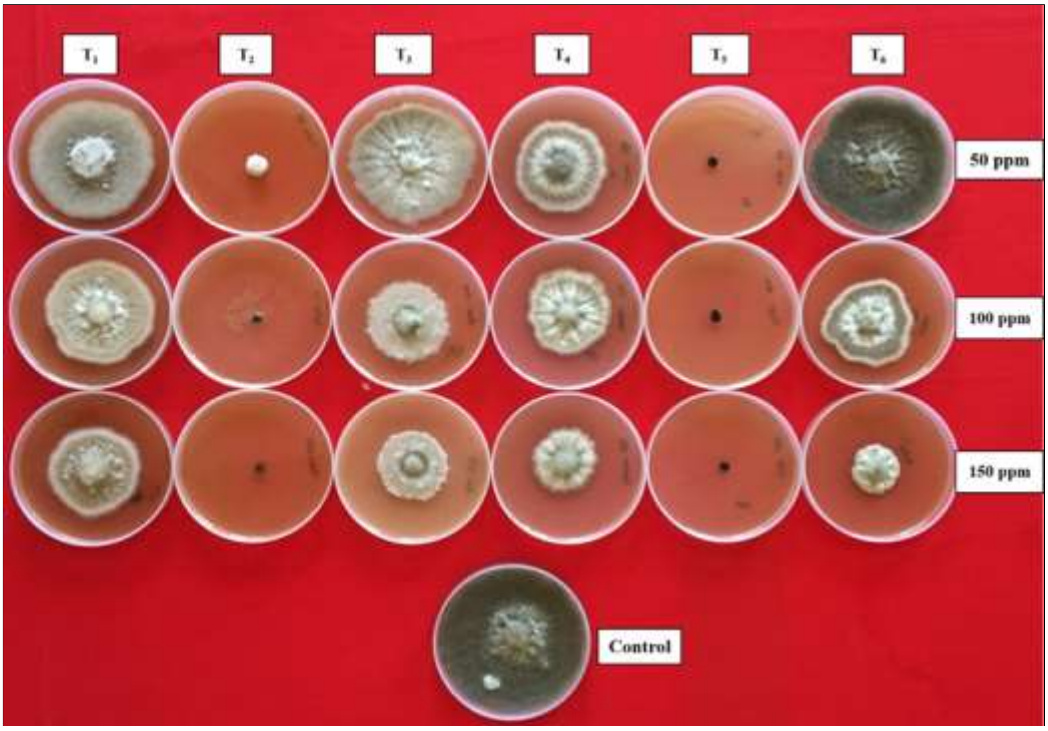
Efficacy of six systemic fungicides *viz.,* thiophanate methyl, propiconazole, carbendazim, azoxystrobin, tebuconazole and tricyclazole were tested against *B. setariae* at three different concentrations (50, 100 and 150 ppm). Obtained data is presented in table 3, fig. 2 and plate 2 revealed that, out of six fungicides, propiconazole showed highest (100 %) significant inhibition of mean mycelial growth and next by tebuconazole (94.69 %) where rest of fungicides gave growth inhibition ranged 28.93-49.96 per cent. Tricyclazole exhibited lowest per cent inhibition (28.93 %). Among the three different concentrations, 150 ppm (59.72 %) showed maximum and 50 ppm (38.07 %) showed lowest per cent inhibition of mean mycelial growth.

**Table 3:** *In vitro* efficacy of systemic fungicides against *B. setariae* infecting browntop millet

| Sl. No. | Fungicide | Per cent inhibition over control* | | | Mean | Microscopic observation |
|---|---|---|---|---|---|---|
| | Concentration | 50 ppm | 100 ppm | 150 ppm | | |
| 1 | Thiophanate methyl | 5.92 (14.08) | 44.07 (41.60) | 64.07 (53.17) | 38.02 (36.28) | Sporulation (+) |
| 2 | Propiconazole | 100.00 (89.71) | 100.00 (89.71) | 100.00 (89.71) | 100.00 (89.71) | No growth |
| 3 | Carbendazim | 38.14 (38.14) | 49.63 (44.79) | 62.09 (52.00) | 49.96 (44.98) | Sporulation (+) |
| 4 | Azoxystrobin | 22.59 (28.38) | 43.82 (41.45) | 50.00 (45.00) | 38.80 (38.28) | Hyphal bulging irregularly at branches |
| 5 | Tebuconazole | 84.07 (66.48) | 100.00 (89.71) | 100.00 (89.71) | 94.69 (81.97) | Slime growth |
| 6 | Tricyclazole | 15.80 (23.42) | 29.14 (32.67) | 41.86 (40.31) | 28.93 (32.13) | Hyphal bulging and Sporulation (-) |
| | Mean | 38.07 (37.21) | 52.38 (48.60) | 59.72 (52.88) | 58.4 | |
| | | Fungicide (F) | Concentration (C) | | F × C | |
| | S.Em ± | 0.11 | 0.07 | | 0.20 | |
| | CD (P 0.01) | 0.30 | 0.20 | | 0.53 | |

**Note**: * Mean of three replications; -: No sporulation; +: 1-15 conidia per microscopic field; Figures in parenthesis are angular transformed values.

With regard to the fungicide and concentration interaction effect, 100 per cent inhibition was recorded in propiconazole at all the (50, 100 and 150 ppm) concentrations and in tebuconazole at 100 ppm and 150 ppm. The lowest (5.92 %) mycelial inhibition was recorded in thiophanate methyl at 50 ppm. Propiconazole and tebuconazole were effective even at lower concentration than other tested fungicides.




**Treatment Details**
$T_1$- Thiophanate methyl 70 % WP
$T_2$- Propiconozole 25 % EC
$T_3$- Carbendazim 50 % WP
$T_4$- Azoxystrobin 23 % SC
$T_5$- Tebuconozole 25 % EC
$T_6$- Tricyclazole 75 % WP

**Plate 2:** *In vitro* efficacy of different systemic fungicides against *B. setariae*

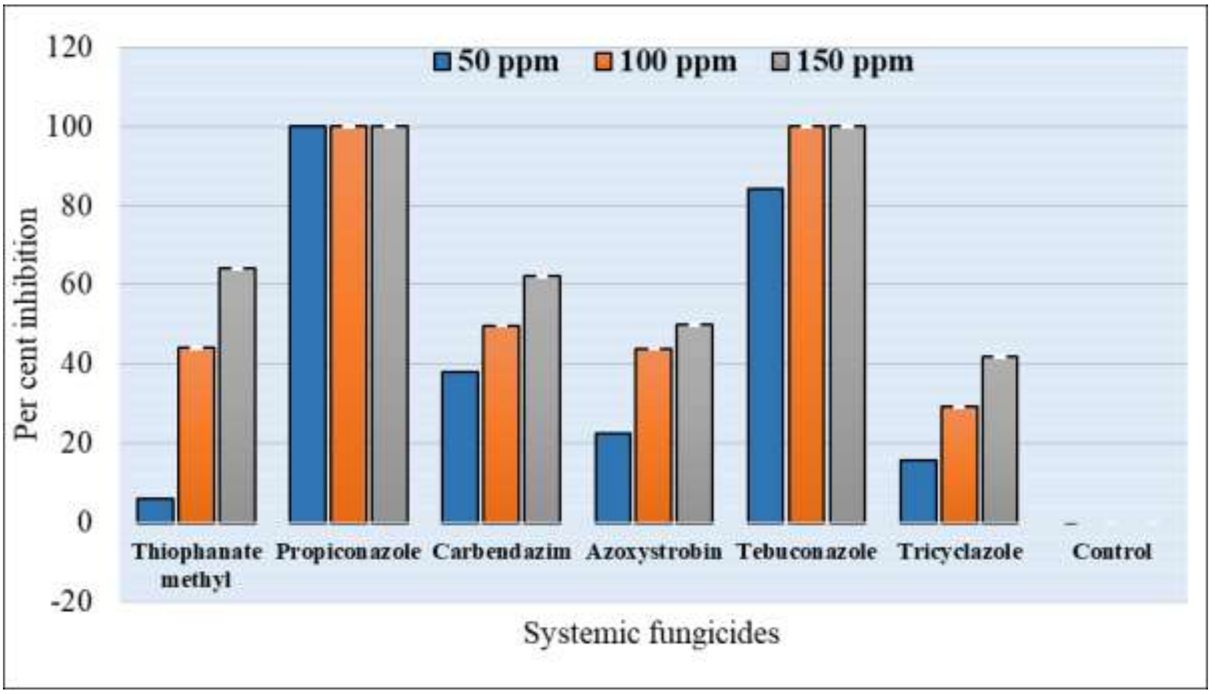



**Fig 2:** *In vitro* efficacy of systemic fungicides on mycelial growth inhibition of *B. setariae*

## Efficacy of combi product fungicides

Results of six combi-product fungicides tested for their efficacy against *B. setariae* at three (100, 250 and 500 ppm) concentrations (Table 4, Fig. 3 and Plate 3).

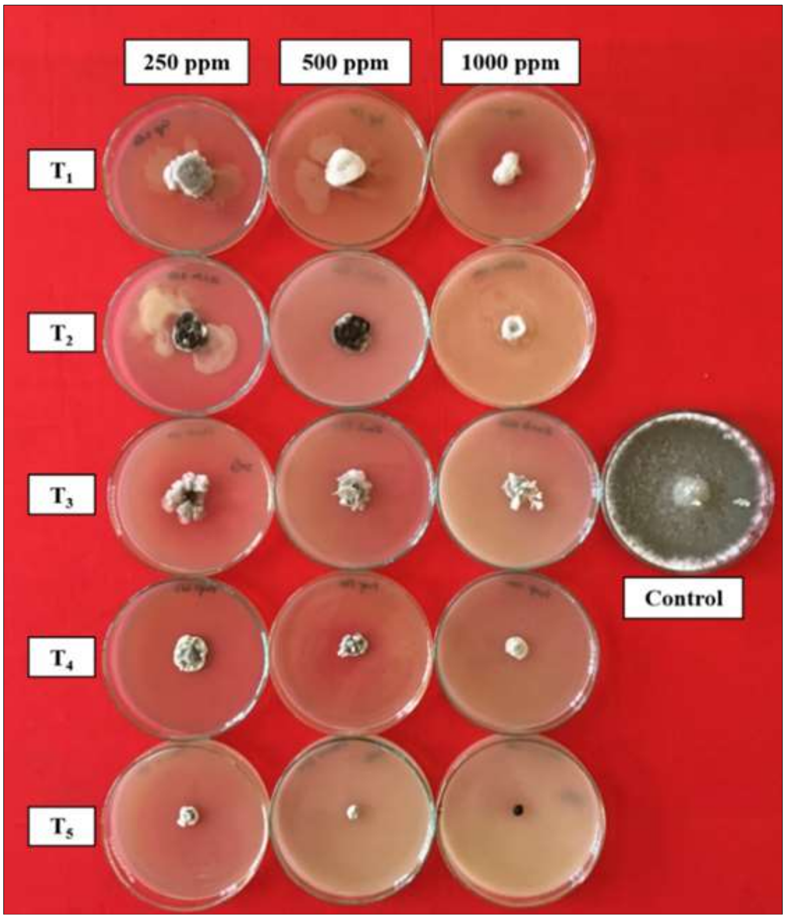
**Table 4:** *In vitro* efficacy of combination product fungicides against *B. setariae* infecting browntop millet

| Sl. No. | Fungicide | Per cent inhibition over control* | | | Mean | Microscopic observation |
|---|---|---|---|---|---|---|
| | Concentration | 100 ppm | 250 ppm | 500 ppm | | |
| 1 | Azoxystrobin + Tebuconazole | 67.45 (55.21) | 70.98 (57.41) | 74.94 (59.96) | 71.13 (57.53) | Slime growth |
| 2 | Cymoxanil + Mancozeb | 60.86 (51.27) | 76.17 (60.78) | 100.00 (89.71) | 79.01 (67.25) | Sporulation (+) |
| 3 | Tricyclazole + Mancozeb | 52.22 (46.27) | 60.12 (50.84) | 100.00 (89.71) | 70.78 (62.27) | Sporulation (-) |
| 4 | Tebuconazole + Trifloxystrobin | 61.85 (51.85) | 72.47 (58.35) | 77.78 (61.87) | 70.70 (57.36) | Slime growth |
| 5 | Carbendazim + Mancozeb | 59.63 (50.55) | 67.77 (55.41) | 100.00 (89.71) | 75.80 (65.22) | Sporulation (+) |
| 6 | Azoxystrobin + Difenoconazole | 64.19 (53.24) | 70.86 (57.33) | 75.18 (60.12) | 70.08 (56.90) | Hyphal bulging and Sporulation (-) |
| | Mean | 52.31 (44.10) | 59.77 (48.63) | 75.41 (64.48) | 72.915 | |
| | | Fungicide (F) | Concentration (C) | | F × C | |
| | S.Em ± | 0.13 | 0.08 | | 0.22 | |
| | CD (P 0.01) | 0.31 | 0.20 | | 0.53 | |

**Note**: * Mean of three replications; -: No sporulation; +: 1-15 conidia per microscopic field; Figures in parenthesis are angular transformed values.

Among the combi fungicides, cymoxanil + mancozeb (79.01 %) showed significant per cent inhibition of mean mycelial growth where lowest per cent inhibition was exhibited by azoxystrobin + difenoconazole (70.08 %). In the other fungicides, mean inhibition ranged 70.70-75.80 per cent. With respect to different concentrations, 500 ppm (75.41 %) recorded maximum and 100 ppm (52.31 %) showed the least per cent inhibition of mean mycelial growth. In the interaction effect of fungicides and concentrations, complete inhibition was recorded in cymoxanil + mancozeb, tricyclazole + mancozeb and carbendazim + mancozeb all at 500 ppm. While other interaction results ranged 52.22-77.75 per cent inhibition. Lowest (52.22 %) was recorded in tricyclazole + mancozeb at 100 ppm.

Efficacy of all (Contact, systemic and combi product) the groups of fungicides on *B. setariae* growth inhibition was achieved by different means *viz.*, inhibition of sporulation, spore germination and hyphal bulging at regular and irregular intervals and also twisting of hyphae that resulted in less growth.




**Treatment Details**
$T_1$- Azoxystrobin 11 % + Tebuconazole 18.3 % SC
$T_2$- Cymoxanil 8 % + Mancozeb 64 % WP
$T_3$- Tricyclazole 18 % + Mancozeb 62 % WP
$T_4$- Tebuconazole 50 % WG + Trifloxystrobin 25 % WG
$T_5$- Mancozeb 63 % + Carbendazim 12 % WP
$T_6$- Azoxystrobin 20 % + Difenoconazole 12.5 % SC

**Plate 3:** *In vitro* efficacy of different combi product fungicides against *B. setariae*

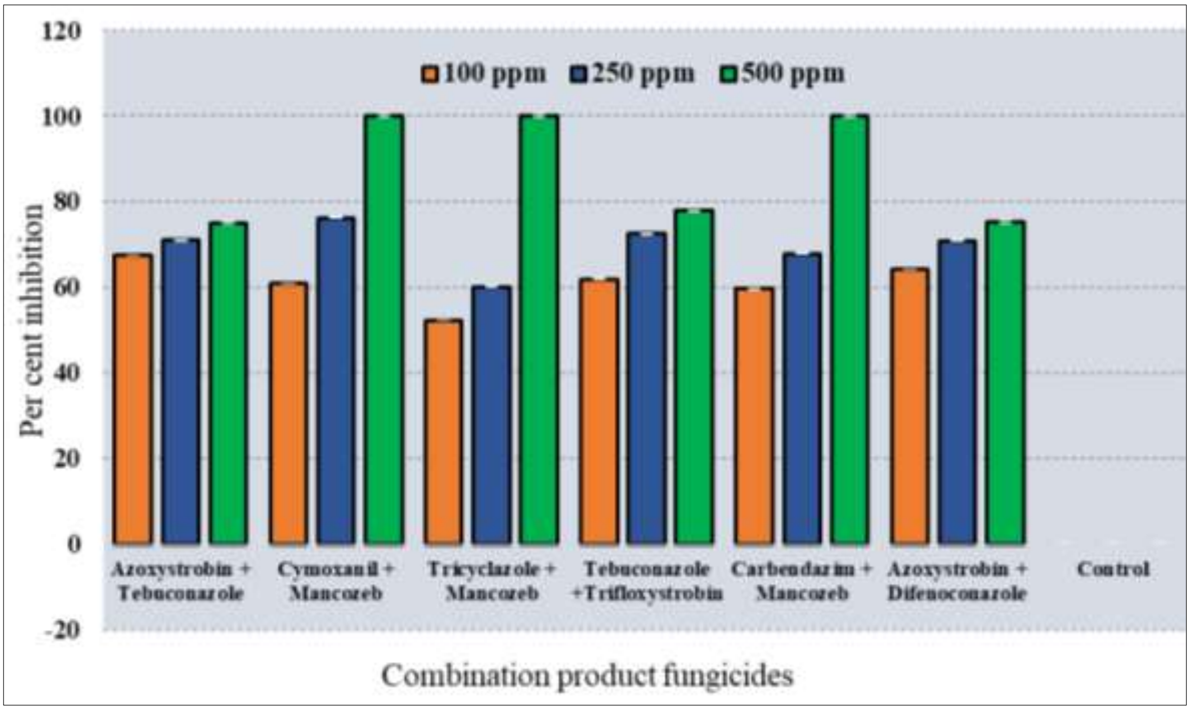



**Fig 3:** *In vitro* efficacy of combi product fungicides on mycelial growth inhibition of *B. setariae*

Tebuconazole, propiconazole and mancozeb fungicides were proved to be effective against the *B. setariae* either as single product or as combination product. Results are in accordance with Kumar *et al.* (2009a), Channakeshava and Pankaja (2018), Mane *et al.* (2018), Nayak and Hiremath (2019), Kavita *et al.* (2017), Harish *et al.* (2017), Meli and Kulkarni (1994) and Gupta *et al.* (2013) [6-13] who reported that, propiconazole was effective against various *Helminthosporium* spp. While Harlapur *et al.* (2007b) [14] found propiconazole, mancozeb and SAAF inhibiting *E. turcicum* growth, Kumar and Chandan (2018) [15] reported proipiconazole and mancozeb as effective against *H. maydis*. Nasir *et al.* (2012) and Nene and Thapliyal (1982) [16, 17] found SAAF and mancozeb were effective against *B. maydis*. Khamari (2014) [18] observed that maximum inhibition of *H. maydis* by cymoxanil 8 % WP + mancozeb 64 % WP followed by mancozeb 63 % WP + carbendazim 12 % WP. Sahoo and Sudipta (2018) [19] noticed tebuconazole 25 EC (95.10 %) as effective against *H. vignicola*. Yamaguchi and Mutsunobu (2010) [20] showed that *Bipolaris*, *Drechslera* and *Exserohilum* were resistant to thiophanate methyl at 100 ppm. Bowen and Pedesen (1988) [21] showed that propiconazole failed totally to inhibit conidial germination of *E. turcicum* which is not so in the present study.

## Conclusion
Among contact fungicides, mancozeb exhibited maximum significant inhibition of mean mycelial growth. Whereas, absolute mycelial inhibition of growth was observed in mancozeb at concentrations of 500 ppm and 1000 ppm. In systemic fungicides, propiconazole showed highest significant inhibition of mean mycelial growth and next by tebuconazole. Cent percent growth inhibition was observed in propiconazole at all (three) the concentrations and in tebuconazole at 100 ppm and 150 ppm. Among the combination fungicides, cymoxanil + mancozeb showed significant per cent inhibition of mean mycelial growth whereas minimal per cent inhibition was exhibited by azoxystrobin + difenoconazole. The results obtained from this study is having vital importance as there is no information available on efficacy of fungicides on *B. setariae* causing leaf blight on browntop millet. However, the field efficacy of these fungicides needs to be evaluated under disease hot spots.

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