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# Efficacy of fungicides and biocontrol agents against *Alternaria alternata*, the causal agent of leaf spot of okra

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There are number of fungicides which are found to be effective for the control of okra leaf spot as reported by several workers. However, there is a need to test newly developed systemic and non-systemic fungicides under laboratory (*in vitro*) and field conditions (*in vivo*) for the effective management of okra leaf spot.Results indicated that all fungicides were completely inhibitory against the mycelial growth of *Alternaria alternata* under *in vitro* condition. Whereas, fungicidesi.e. Tebuconazole 50% + Trifloxystrobin 25%WG (61.27 %), Azoxystrobin 8.2% + Difenoconazole 11.4% SC (57.83%), Metiram 55%+Pyraclostribin 5% WG (57.04 %), Azoxystrobin 11%+Tebuconazole 18.3% SC (56.16 %), Hexaconazole 4%+Zineb 68% WP (56.16%) and Iprobenfos 48% EC (54.27%) were found significantly effective in reducing per cent disease index (PDI) of leaf spot of okra over control. Amongst biocontrol agents, stains of *Bacillus subtilis* (65.03%- 59.06%) were found more effective in controlling the leaf spotof okra.

**Keywords :** *Bacillus subtilis,* Difenoconazole,Hexaconazole, Propiconazole

## INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is a nutritious and delicious annual vegetable crop, belongs to Malvaceae family and grown in tropical and subtropical climates (Vasava and Patel (2020). The total area grown in India is 5.09 lakhs hectares, having productivity of 12.0 million tons per hectare with annual production of 60.95 lakhs tones. Chhattisgarh is the leading producer followed by Orissa, Madhya Pradesh, Bihar, West Bengal and Gujarat. Okra covers 32.60 thousand hectares of cultivated Area in Chhattisgarh with a total production of 3.59 lakhs metric tons and a productivity of 11.01 metric tons/ha (Anon., 2023). Among several diseases i.e. downy mildew, powdery mildew, leaf spot, mosaic, root knot etc. caused by fungi, bacteria, viruses and nematodes are responsible for reduction in fruit yield of okra crops (Thippeswami*et al*., 2007 and Choudhary and Panchouli, 2024). *Alternaria* leaf spot of okra caused by *Alternaria alternata*is one of the most destructive disease, reported from various parts of world and causes losses upto 30-50% or more in fruit yield of okra (Thippeswamy *et al.,* 2007; Pansambal *et al.,* 2015, Sindhumole *et al.,* 2015).

Based on damage nature and survival capability of the fungus, the use of chemicals for management of leaf spot of okra was considered to be the only economical and practical solution (Choudhary and Panchouli, 2024). There are number of fungicides which were found to be effective for the control of okra leaf spot as reported by several workers (Hurule *et al*. 2019). However, there is a need to test newly developed systemic and non-systemic fungicides under laboratory and field conditions for the effective management of okra leaf spot.Newer generation fungicides offer several advantages in controlling Alternaria leaf spot of okra, including enhanced efficacy, broader spectrum of activity, and reduced risk of resistance development. They are often more potent, requiring lower application rates, and may have improved environmental profiles compared to older fungicides (Sajad *et al*. 2017; Gandhi and Rao, 2019). Biological control of plant diseases with microbial antagonists has been emerging as one of the potential alternatives. Different workers have reported the effectiveness of biocontrol

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agents *i.e. Trichoderma viride, Pseudomonas fluorescens and Bacillus subtilis* against diseases caused by *Alternaria* species (Hurule *et al*. 2019,Raut *et al*.2020*).*Therefore, present study was undertaken to evaluate new formulations of fungicides as well as liquid formulations of biocontrol agents for the effective management of leaf spot of okra.

## MATERIALS AND METHODS

### *Isolation of pathogen Alternaria alternata from infected plant*

The infected disease sample leaves of okra plants were collected from okra field. These were washed for five minutes under running tap water to remove dirt and dust particles and completely dried using paper towel method. The leaves were cut into small pieces that ranges from 0.2-0.5 cm, these were then surface disinfected in 1% sodium hypochloride solution for one minute and washed thrice in sterilized distilled water to remove chemical traces and again dried completely to avoid surface contamination. 5 pieces were placed in petri plates having potato dextrose agar medium. The whole process was carried out with great care under aseptic conditions. The dishes were incubated at 28±1°C for mycelial growth and sporulation of the test pathogen. The pure culture was developed through subculture technique (Arain *et al*. 2012).

### *Evaluation of the efficacy of fungicides against the mycelial growth of Alternaria alternata under in vitro condition*

The efficacy of fungicidesi.e.Propiconazole 25% EC, Azoxystrobin 11% + Tebuconmazole 18.3% SC, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 4% + Zineb 68% WP, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Metiram 55% + Pyraclostribin 5% WG, and Iprobenfos 48% EC) were evaluatedat different concentrations i.e. 100, 500, 1000, 1500 and 2000 ppm against the mycelial growth of *Alternaria alternata* using poisoned food technique (Nene and Thapliyal, 1982).

Required quantity of each fungicide was added separately to sterilized medium mixed thoroughly and poured in sterilized petri plates and allowed to solidify. Each plate was inoculated with 5 mm disc of 10-12 days old culture of *Alternaria alternata* with the help of sterilized cork borer and incubated at 28 $\pm$ 2°C for 10-12 days. A control was maintained where medium was not supplemented with any fungicides. The experiment was conducted in completely randomized design with three replications.Radial mycelial growth (mm) of test pathogen mentioned above was recorded from each treatment after 2 days (48 hrs.), 4 days (96 hrs.), 6 days (144 hrs.), 8 days (192 hrs.), 10 days (240 hrs.) and 12 days (288 hrs.) of incubation.

The percentage inhibition of mycelial growth was calculated using the formula developed by Vincent (1947)

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

I = per cent inhibition
C = Mycelial growth in control
T = Mycelial growth in treatments

### *Evaluation of the efficacy of fungicides for the control of leaf spot ofokra under natural epiphytotic condition*

**Treatment details-**

Treatments - 8 (fungicides + control)
Plot size – 2mts x 1mts
Design – Randomized block design

Field experiment was conducted during *Rabi* season in 2023-2024.The plots were prepared by tillage operations followed by tractor drawn disc harrowing and planking. Thereafter, the experiment was laid out as per plan and design. The seeds of okra variety “Green wonder” were sown manually using line sowing method with distance of 60cm X 20cm between row and plant respectively.

After the appearance of first symptoms of leaf spot, different treatments *were* applied as foliar application on the host plants covering all aerial parts of plant above ground level. Two sprays were applied at an interval of 10 days. Pre-treatment observations were recorded first whereas, post treatment observations were recorded at 10 days after the completion of first

| Treatments | Fungicides | Dose (ml, g/l water) |
|---|---|---|
| T1 | Propiconazole 25% EC | 2ml |
| T2 | Azoxystrobin 11% + Tebuconazole 18.3% SC | 1.5ml |
| T3 | Tebuconazole 50% + Trifloxystrobin 25% WG | 0.5 gm |
| T4 | Hexaconazole 4% + Zineb 68% WP | 3gm |
| T5 | Azoxystrobin 18.2% + Difenoconazole 11.4 SC | 1ml |
| T6 | Metiram 55% + Pyraclostrobin 5% WG | 1.5gm |
| T7 | Iprobenfos 48% EC | 2ml |
| T8 | Control | - |

| Treatments | Biocontrol agents | Dose ml/l water |
|---|---|---|
| T1 | *Bacillus subtilis* strain 1 | 10 ml |
| T2 | *Bacillus subtilis* strain 2 | 10 ml |
| T3 | *Bacillus subtilis* strain 3 | 10 ml |
| T4 | *Bacillus subtilis* strain 4 | 10 ml |
| T5 | *Bacillus subtilis* strain 5 | 10 ml |
| T6 | *Bacillus subtilis* strain 6 | 10 ml |
| T7 | *Bacillus subtilis* strain 7 | 10 ml |
| T8 | *Bacillus subtilis* strain 8 | 10 ml |
| T9 | *Pseudomonas fluorescens* | 10 ml |
| T10 | *Trichoderma viride* | 10 ml |
| T11 | Hexaconazole 4% + zineb 68% WP | 3g |
| T12 | Control | - |

spray and second observation was recorded after 10 days of second spray.The plants were observed critically for visual scoring of disease severity using the standard evaluation scale (0-5 scale) given by Hurule *et al*. (2019).

***Evaluation of the efficacy of biocontrol agents for the control of leaf spot of okra under natural epiphytotic condition***

**Treatment details-**

Treatments - 12 (biocontrol agents + control)
Plot size –2mts x 1mts
Design – Randomized block design

***Liquid formulation of biocontrol agents***

Liquid formulation of *Pseudomonas fluorescens, Trichoderma virideand* different strains of *Bacillus subtilis* were procured from SBCL, BTC CARS, Bilaspur, IGKV (C.G.). Foliar application of all the treatments was done on okra field.

***Treatment application***

After the appearance of first symptoms of leaf spot, different treatments *i.e.Pseudomonas fluorescens* (PF), *Trichoderma viride* and different strains of *Bacillus spp.* were applied as foliar application on the host plants covering all aerial parts of plant above ground level. Two sprays were applied at an interval of 10 days. Pre-treatment observations were recorded first whereas, post treatment observations were recorded at 10 days after the completion of first spray and second observation was recorded after 10 days of second spray.The plants were observed critically for visual scoring of disease severity using the standard evaluation scale (0-5 scale) given by Hurule *et al*. (2019).

***Observations recorded***

The disease severity of leaf spot of okra was recorded from both the experiments under natural epiphytotic condition. Pre-treatment observations of leaf spot of okra was taken from different plots prior to application of fungicides The plants were observed critically for visual scoring of disease severity using the standard evaluation scale (0-5 scale) given by Hurule *et al*.(2019).

Per cent disease index (PDI) was calculated by using the formulas given by (Hurule *et al*., 2019).

| 0-5 scale | Per cent leaf area affected |
|---|---|
| 0 | No infection |
| 1 | 0.1-5% area covered with the pathogen |
| 2 | 5.1-20% area covered with the pathogen |
| 3 | 20.1-50% area covered with the pathogen |
| 4 | 50.1-75% area covered with the pathogen |
| 5 | >75% area covered with the pathogen |

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of numerical ratings observed}}{\text{No of leaves per plants observed x Maximum disease scale}} \times 100$$

Reduction (%) Per cent disease index (PDI)=

$$\frac{C - T}{C} X\ 100$$

Where, C = Per cent disease index (PDI) in control plots
T = Per cent disease index (PDI) in treated pots

## RESULTS AND DISCUSSION

### *Efficacy of fungicides against the mycelial growth of Alternaria alternata under in-vitro condition*

Data presented in Table1and Fig. 1indicate that all fungicides were significantly effective in checking the mycelial growth of *Alternaria alternata* (38.00 mm – 71.50 mm) over untreated control (90.00 mm) at different concentrations. However, fungicides *i.e.* Propiconazole 25% EC, Azoxystrobin 11% + Tebuconazole 18.3% SC, Hexaconazole 4%+Zineb 68% WP, Azoxystrobin 18.2%+ Difenconazole 11.4% SC were found completely inhibitory even at 100ppm concentration showing high potential against *Alternaria alternata* compared to other fungicides.

The higher efficacy of systemic and combo fungicides (combination of two molecules) under the present study might be due to synergistic action of both molecules in inhibiting the mycelial growth of *Alternaria alternata*. The results of present study are in accordance with the findings of Hurule *et al*. (2019), Kokare *et al*. (2020), Deshmukh *et al*. (2020) and Ghule *et al.* (2023) who reported that the systemic fungicides i.e. Hexaconazole 5% EC, Tebuconazole 25.9% EC, Difenconazole 25% EC, Propiconazole 25% EC and combined formulations of fungicides i.e. Tebuconazole 50% + Trifloxystrobin 25% WG, Azoxystrobin + Difenconazole were found most effective and recorded complete inhibition of growth of the *Alternaria alternata*.

### *Efficacy of fungicides and biocontrol agents for the control of leaf spot of okra under natural epiphytotic condition*

Field experiments on the efficacy of fungicides and biocontrol agents were conducted in *Rabi* season using randomized block design for the management of the leaf spot of okra.Data presented in Table2 and Fig.2 of per cent reduction of leaf spot severity over control indicated that all fungicides were found significantly effective in reducing the leaf spot disease of okra (46.69 %–61.27%) over control (78.67%) . However,fungicidesi.e. Tebuconazole 50%+ Trifloxystrobin25%WG (61.27 %), Azoxystrobin 8.2% + Difenoconazole 11.4% SC (57.83%), Metiram 55%+Pyraclostribin 5% WG (57.04 %), Azoxystrobin 11% +Tebuconazole 18.3% SC (56.16 %), Hexaconazole 4% + Zineb 68% WP (56.16%) and Iprobenfos 48% EC (54.27%)were found highly effective in controlling the leaf spot disease of okra.The results of present study are in accordance with the findings of Kumar and Singh, (2020) who reported the minimum per cent disease intensity of leaf spot of okra with maximum disease control in the treatments *i.e.* Tebuconazole 25% + Trifloxistrobin 50% WG followed by Azoxystrobin 18.2% + Difeconazole 11.4% SC, Tebuconazole 25 EC, and Propiconazole 25 EC. The new generation of systemic fungicides especially the formulations having combination of two molecules used under the present study were found to be more effective in controlling the leaf spot severity over other fungicides which are already in recommendation for the management of leaf spot of okra.

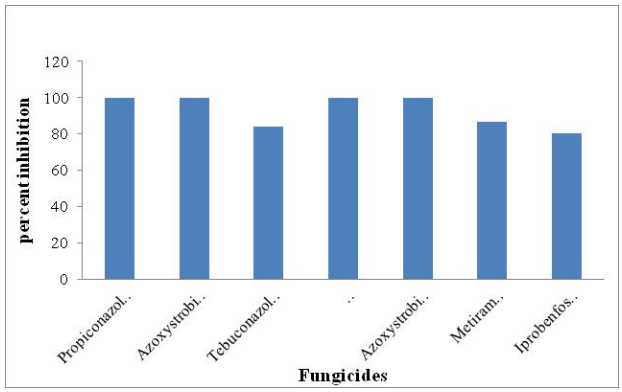


**Fig 1** :*In vitro* evaluation of fungicides on percent inhibition of mycelial growth of *Alternaria alternata*

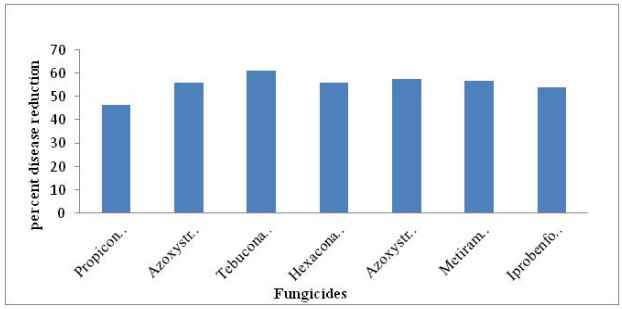


**Fig 2 :** Per cent reduction on the severity of *Alternaria* leaf spot of okra by the foliar application of fungicides

**Table 1.** *Invitro* evaluation of fungicides at different concentrations (ppm) against mycelial growth of *Alternaria alternata*

| Treatments (Fungicides) | Radial mycelial growth (mm) | | | | | |
|---|---|---|---|---|---|---|
| | 100ppm | 500ppm | 1000ppm | 1500ppm | 2000ppm | Mean |
| $T_1$-Propiconazole 25% EC | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 |
| $T_2$-Azoxystrobin 11% + Tebuconazole 18.3% SC | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 |
| $T_3$-Tebuconazole 50% + Trifloxystrobin 25% WG | 71.50 (57.73) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 14.30 |
| $T_4$- Hexaconazole 4% + Zineb 68% WP | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 |
| $T_5$-Azoxystrobin 18.2% + Difenconazole 11.4% SC | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 0.00 |
| $T_6$-Metiram 55% + Pyroclostrobin 5% | 38.00 (38.05) | 20.83 (27.15) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 11.77 |
| $T_7$-Iprobenfos 48% EC | 46.00 (42.70) | 43.17 (41.07) | 0.00 (0.26) | 0.00 (0.26) | 0.00 (0.26) | 17.83 |
| $T_8$-Control | 90.00 (71.56) | 90.00 (71.56) | 90.00 (71.56) | 90.00 (71.56) | 90.00 (71.56) | 90.00 |
| Mean | 30.69 | 19.25 | 11.25 | 11.25 | 11.25 | |
| C.D. 5% - Fungicide – 0.22 | Concentration – 0.17 | | F x C – 0.49 | | | |
| CV % - 2.14 | | | | | | |

*Figures in the parentheses indicate arc sine transformed; Incubation Period- 12 days

Results on per cent reduction in leaf spot severity over control indicated that all biocontrol agents i.e. *Pseudomonas fluorescence*, *Trichoderma viride* and different stains of *Bacillus subtilis* were significantly effective in reducing the severity (46.03 %- 75.16%) of leaf spot disease of okra over control (Table 3 and Fig 3). However, some stains of *Bacillus subtilis* (65.03%- 59.06%) were found more effective in controlling the leaf spotof okra. The results of present study are in accordance with the findings of Priyanka *et al*. (2018) and Raut *et al*. (2020) who reported that *Pseudomonas fluorescence* and *B. subtilis* subsp. *spizizenii* were found effective in the management of early blight disease of tomato caused by *Alternaria solani* with PDI of 33.16% over control (PDI-42.40).

## CONCLUSION

It may be suggested that Tebuconazole 50% + Trifloxystrobin 25% WG, found highly effective in the present study can be used as foliar spray during the vegetative stage of the crop, followed by foliar spray of *Bacillus subtilis* during the flowering and fruiting stages of okra crop for the control of leaf spot disease of okra and thus residual effect of fungicides on okra fruit can be avoided.

## ACKNOWLEDGEMENTS

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**Table 2** : Efficacy of fungicides as foliar spray for the management of leaf spot of okra caused by *Alternaria alternata*

| Treatments (Fungicides) | Concentration/ dose (g ml$1^{-1}$) | Percent Disease Index (PDI) | | | % Reduction over control | |
|---|---|---|---|---|---|---|
| | | Before spray | 10days After 1 st spray | 10days After 2nd spray | 10days After 1 st spray | 10days After 2nd spray |
| $T_1$-Propiconazole 25% EC | 2ml/l | 50.67 (45.40) | 50.00 (45.00) | 42.00 (40.38) | 31.29 (33.95) | 46.69 (43.1) |
| $T_2$-Azoxystrobin 11% + Tebuconazole 18.3% SC | 1.5ml/ l | 40.80 (39.64) | 40.67 (39.53) | 34.67 (36.01) | 44.29 (41.63) | 56.16 (48.55) |
| $T_3$-Tebuconazole 50% + Trifloxystrobin 25% WG | 0.5gm/l | 38.80 (38.39) | 36.00 (36.70) | 30.67 (33.55) | 50.53 (45.30) | 61.27 (51.53) |
| $T_4$-Hexaconazole 4% + zineb 68% WP | 3gm/l | 58.40 (49.87) | 42.67 (40.77) | 34.67 (35.93) | 41.43 (40.02) | 56.16 (48.55) |
| $T_5$-Azoxystrobin 18.2% + Difenoconazole 11.4 SC | 1ml/l | 47.33 (43.46) | 40.67 (39.45) | 33.33 (35.11) | 44.29 (41.63) | 57.83 (49.51) |
| $T_6$-Metiram 55% + Pyraclostrobin 5% WG | 1.5gm/l | 39.20 (38.60) | 45.33 (42.30) | 34.00 (35.49) | 37.79 (37.83) | 57.04 (49.06) |
| $T_7$-Iprobenfos 48% EC | 2ml/l | 50.67 (45.36) | 43.47 (40.94) | 36.00 (36.85) | 40.31 (39.36) | 54.27 (47.44) |
| $T_8$- Control | - | 62.67 (52.34) | 72.67 (58.49) | 78.67 (62.81) | 0.00 (0.58) | 0.00 (0.58) |
| C.D. 5% | - | NS | 7.41 | 7.62 | - | - |
| C.V. % | | NS | 9.86 | 11.02 | | |

*Figures in the parentheses indicate arc sine transformed

**Table 3**. Efficacy of biocontrol agents as foliar spray for the management of leafspot of okra caused by *Alternaria alternata*

| Treatments | Doses/liter | Percent Disease Index (PDI) | | | % reduction over control | |
|---|---|---|---|---|---|---|
| | | before spray | 10days After 1st spray | 10days After 2nd spray | 10days After 1st spray | 10days After 2nd spray |
| $T_1$-B. *subtilis*-1 (Bs-1) | 10ml | 46.27 (42.85) | 40.00 (39.22) | 32.67 (34.85) | 43.30 (41.13) | 59.06 (50.22) |
| $T_2$- B. *subtilis*-2 (Bs-2) | 10ml | 40.67 (39.58) | 34.67 (36.01) | 30.67 (33.55) | 51.09 (45.62) | 61.85 (51.89) |
| $T_3$- B. *subtilis*-3 (Bs-3) | 10ml | 45.73 (42.54) | 32.00 (34.22) | 29.33 (32.74) | 54.47 (47.70) | 63.11 (52.66) |
| $T_4$- B. *subtilis*-4 (Bs-4) | 10ml | 44.40 (41.77) | 38.00 (38.02) | 31.33 (34.02) | 46.08 (42.73) | 60.72 (51.21) |
| $T_5$- B. *subtilis*-(Bs-5) | 10ml | 40.67 (39.62) | 38.00 (38.02) | 32.00 (34.42) | 46.13 (42.77) | 59.85 (50.71) |
| $T_6$- B. *subtilis*-(Bs-6) | 10ml | 44.40 (41.78) | 41.33 (40.00) | 37.33 (37.62) | 41.39 (40.02) | 53.75 (47.17) |
| $T_7$- B. *subtilis*-(Bs-7) | 10ml | 46.00 (42.70) | 37.33 (37.63) | 28.00 (31.91) | 47.00 (43.26) | 65.03 (53.77) |
| $T_8$- B. *subtilis*-(Bs-8) | 10ml | 47.73 (43.69) | 48.93 (44.38) | 43.33 (41.14) | 30.78 (33.67) | 46.03 (42.70) |
| $T_9$-*Pseudomonas fluorescens* | 10ml | 46.00 (42.70) | 44.40 (41.73) | 37.33 (37.51) | 37.35 (37.03) | 53.75 (47.17) |
| $T_{10}$-*Trichoderma viride* | 10ml | 50.00 (45.00) | 46.00 (42.68) | 37.33 (37.51) | 35.13 (35.66) | 53.75 (47.17) |
| $T_{11}$-Hexaconazole 4% + zineb 68% WP | 3g | 43.33 (41.13) | 30.00 (33.15) | 20.00 (26.44) | 57.57 (49.38) | 75.16 (6019) |
| $T_{12}$- Control | - | 54.67 (47.75) | 70.67 (57.21) | 80.00 (63.50) | 0.00 (0.47) | 0.00 (0.47) |
| C.D. 5% | | NS | 7.74 | 6.66 | - | - |

*Figures in the parentheses indicate arc sine transformed

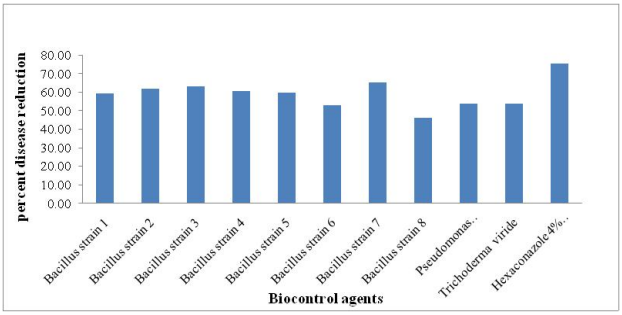


**Fig 3.** Per cent reduction on the severity of *Alternaria* leaf spot of okra by the foliar application of biocontrol agents.

## DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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