In vitro assessment of fungicides against *Alternaria alternata* causing leaf spot of okra

Abstract

The present investigation was undertaken to test the efficacy of some promising fungicides for its effective management against leaf spot of okra under in vitro. Okra is affected by several fungal, bacterial, viral and nematode induced diseases. *Alternaria alternata* attacked old leaves and progress upwards. In initial stage of infection small, scattered, brown colored necrotic spots with concentric rings appeared on older leaves that later enlarged in size and these spots were accompanied by narrow chlorotic margins. Diseased stem and leaves of okra, showing characteristic symptoms of Alternaria blight were collected from the vegetable field of JNKVV, Jabalpur. Based on present investigation it could be concluded that among the Nine chemical fungicides namely Tebuconazole, Hexaconazole, Mencozeb, Copper oxychloride, Carbandazim, Thiram, Propiconazole, Copper oxychloride + Carbandazim and captan + Hexaconazole showed the best result with the maximum inhibition in growth of A. alternata was recorded by Propiconazole (100 %) followed by Tebuconazole (86.02 %) at all the concentrations. This finding will be greatly helpful to okra growers by integrating with different management strategies for the purpose of control of okra leaf spot of okra in field condition which may impart positive impact.

Comment [u1]: Okra is a most demanded vegetable crop; afflicted by a number of diseases, the most serious and destructive of which is leaf spot of okra caused by *Alternaria alternata,* which causes quantifiable losses. In present study evaluated *in vitro* efficacy of nine fungicides. Among the Nine chemical fungicides, Propiconazole recorded maximum growth inhibition (100%) at 100 ppm and was found superior over rest other fungicidal treatments. The next best fungicidal treatment was Tebuconazole (86.02%) followed by Carbendazim (82.35), Copper oxychloride + Carbandazim (72.35), Mencozeb (62.79), Thiram (59.8), which recorded colony diameter of 9.5, 12.3, 18.8, 25.3, 27.33 mm, respectively; while, Copper oxychloride showed least growth inhibition (43.23) of the test pathogen with colony diameter of 40 mm.

Keywords: Fungicides, chlorotic margins, nematode, *Abelmoschus esculentus*

Introduction

Okra,(*Abelmoschus esculentus* (L). Moench belongs to the family Malvaceae, and originated in Abyssinia (Anon, 2008). It is one of the important vegetables, mainly grown for its tender fruits in many countries of the world. India ranks first in area and production of okra and has been commercially grown in the states of Andhra Pradesh, West Bengal, Jharkhand, Orissa, Utter Pradesh, Madhya Pradesh, Karnataka, Gujarat and Maharashtra. The crop occupies nearly 511 thousand hectares area, production 5848.6 thousand metric tones and productivity of 11.40 metric tones/ha. In Madhya pradesh okra crop occupies area 27.11 thousand hectares with production 342.05 thousand metric tones and productivity 12.62 metric tones/ha (Anonymous 2017). Biotic and abiotic stresses under changing climate are major hurdles in profitable production of various crops including okra.

Okra is affected by several fungal, bacterial, viral and nematode induced diseases. Okra crop is being affected by major fungal diseases *viz.*, *Alternaria* leaf spot (*Alternaria alternata* / *A. chlamydospora*), (Thippeswami *et al*., 2007) powdery mildew (*Erisiphe cichoracearum* and *Leveillula taurica*), (Sridhar *et al*.,1989), root / charcoal rot (*Microphomina phaseolina*), wilt (*Fusarium oxysporum* f. sp. *vasinfectum*), Cercospora leaf spot (*Cercospora abelmoschi, C. malayensis*) and damping off (*Pythium sp*., *Rhizoctonia sp*.), and Yellow vein mosaic (YVMV) (Jambhale and Nerkar, 1981). Alternaria leaf spot of okra has been reported from various parts of world (Tohyama *et al*., 1995). And is responsible to cause 30-50 percent yield losses (Thippeswami *et al.* 2007)

In the field, *Alternaria alternata* attacked old leaves and progress upwards. In initial stage of infection small, scattered, brown colored necrotic spots with concentric rings appeared on older leaves that later enlarged in size and these spots were accompanied by narrow chlorotic margins. Finally, the spots coalesced which resulted in production of numerous conidia on dying or dead tissues. These conidia are considered to be major sources of airspora in okra fields (Tohyama *et al.* 1995).

Alternaria alternata propagates itself via asexual spores called conidia. These conidia are produced in lesions on mature or dying leaves. When the conidium lands on a leaf, it will wait until the night time dew, and then germinate. It can either enter through the stomata, or penetrate directly through the top of the leaf, using its appressorium, infecting the leaf within 12 hours (Peter *et al*. 1987).

Use of fungicides is the quickest and effective method for Fusarium wilt management (Moosa *et al.,* 2016). Since many years, control of several fungal diseases is managed by employing the potential of fungicides (Bharat *et al.,* 2006). 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. Keeping in view, the importance and losses caused by leaf spot of okra, present investigation was undertaken to test the efficacy of some promising fungicides for its effective management against leaf spot of okra under *in vitro*.

Materials and Methods

Comment [u2]: Mention the name of fungicides and their concentration

Diseased stem and leaves of okra, showing characteristic symptoms of *Alternaria* blight were collected from the vegetable field of JNKVV, Jabalpur. The leaves were thoroughly washed with tap water and dried with the help of blotter paper. These were then used to isolate the causal agent. The leaf portions were alienated with sterile scalpels and kept in different envelopes. Each envelope was marked clearly mentioning location, variety, date of collection etc. and were brought to the laboratory. The samples were dried for 24 hours in shade in order to remove excess surface moisture. After drying, the samples were kept in B.O.D. incubator in paper envelop and maintained for isolation and further studies.

Isolation and purification of culture

Small pieces measuring one cm, from affected stem and leaves, were cut with the help of sterilized blade. These were later surface sterilized with sodium hypo chloride (1000 ppm) for 30 seconds and removed excess moisture with the help of sterilized blotter paper and placed in Petri plates containing sterilized PDA aseptically. Petri plates were incubated at 28°C $\pm$ 2 for fungal growth and were examined regularly. The casual organism developed was identified as *A. alternata* pure culture was developed from single hyphal tip method. The pure culture so obtained was maintained on PDA slants.

Evaluation of fungicides:

In order to find out a suitable fungicide for management of leaf spot of okra. Nine fungicides, namely Tebuconazole, Propiconazole, Copper oxychloride, Thiram, Carbendazim, Hexaconazole, Mancozeb, COC+ Carbendazim, Captan + Hexaconazole along with control was evaluated against leaf spot of okra in by following the poisoned food technique under *in vitro* condition. PDA poisoned with each fungicide will be poured into three sterilized Petri plates @ 20 ml/plate and allowed to solidify. Plates containing PDA without fungicide served as check. After solidification each Petri plate was inoculated with 5 mm mycelial disc aseptically. Experiment was conducted in Completely Randomized Design (CRD). Each treatment was replicated thrice and the inoculated plates were incubated at 25±2°C in BOD incubator. Observation on radial growth of test fungus will be recorded after 168 hours. Recorded data on radial growth was converted into percent growth inhibition by using following formula:

Percent inhibition (I) = I = (C-T) X100 C

Where,

C = Colony diameter in check plate (mm)

T = Colony diameter in the treated plate (mm)

Results and Discussion

Nine chemical fungicides namely Tebuconazole, Hexaconazole, Mencozeb, Copper oxychloride, Carbandazim, Thiram, Propiconazole, Copper oxychloride + Carbandazim and captan + Hexaconazole at 10, 25, 50 and 100 ppm concentrations were evaluated against *A. alternata.* Propiconazole recorded maximum growth inhibition (100%) at 100 ppm concentration of the test pathogen with minimum colony diameter of 0 mm and was found superior over rest other fungicidal treatments. The next best fungicidal treatment was Tebuconazole (86.02%) followed by Carbendazim (82.35), Copper oxychloride + Carbandazim (72.35), Mencozeb (62.79), Thiram (59.8), captan + Hexaconazole (59.63), Hexaconazole (58.44), which recorded colony diameter of 9.5, 12.3, 18.8, 25.3, 27.33, 27.63, 28.5 mm, respectively; while, Copper oxychloride showed least growth inhibition (43.23) of the test pathogen with colony diameter of 40 mm. Hence, the treatment of fungicides Propiconazole and Tebuconazole was proved most effective in inhibiting the growth of *Alternaria alternata* as compared to other fungicides and untreated control after 120 hrs of incubation. Inhibitory effect of Propiconazole on mycelia growth of *Alternaria alternata* had been reported by (Phapale *et al.* (2010). Propiconazole belong to triazole fungicides which act as demethylase inhibitor (DMI) and interfere in the process of building the structure of fungal cell wall and finally it inhibits the reproduction and growth of the fungus. Azoxystrobin is strobilurin fungicides which interfere with respiration in fungi (Attri *et al.,* 2019). Carbendazim (0.1%) and Tebuconazole (0.1%) were found best solo fungicides and Carboxin + Thiram (0.25%), Carbendazim + Mencozeb (0.2%), and Azoxystrobin + Difenoconazole (0.1%), found best combi fungicides which completely inhibited the radial growth and sporulation of Fusarium udum. (Patel *et al.,* 2021). Present findings are also supported by Arain *et al.* (2012) evaluated six fungicides among them Ridomil MZ found effective for the control of *Alternaria* leaf spot disease of okra in Sindh (Pakistan). Pancholi *et. al.* also reported that, under *in vitro* conditions seven fungicides *viz.,* Azoxystrobin, Hexaconazole, Thiram, Tebuconazole, Pyraclostrobin, Carbendazim, Mancozebalong with control was evaluated against *Fusarium oxysporum* f. sp. *pisi* causal agent of wilt of pea. Tebuconazole and Pyraclostrobin were found best fungicides which completely inhibited the radial growth of *F. oxysporum* f. sp. *pisi.*

Table No. 1. Effect of Fungicides on mycelial growth of *Alternaria alternata* after 120 hours

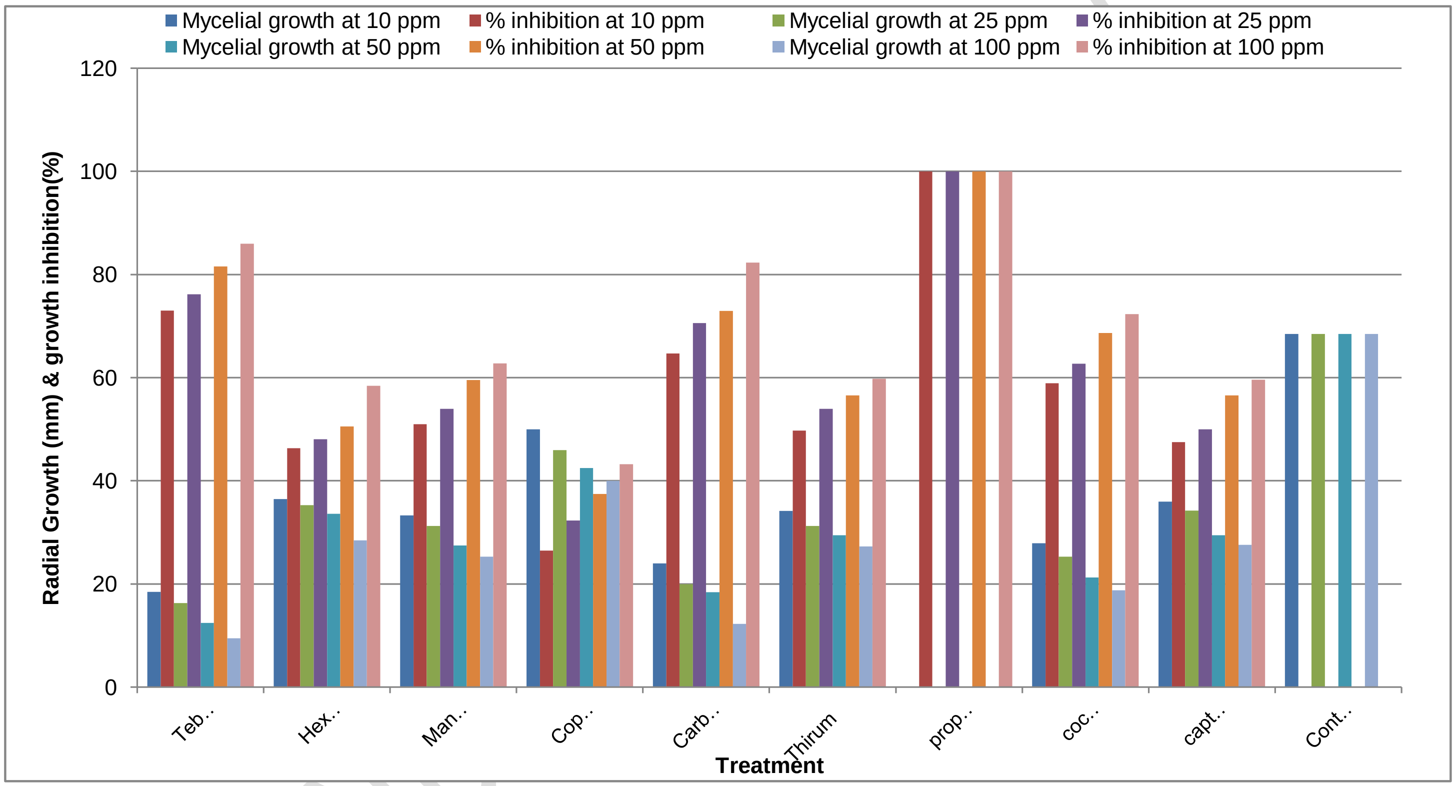
Fungicide	Mean radial growth (mm)							
	Concentration (ppm)							
	10%	**% inhibition**	**25%**	**% inhibition**	**50%**	**% inhibition**	**100%**	**% inhibition**
Tebuconazole	18.5	73	16.3	76.21	12.5	81.61	9.5	86.02
Hexaconazole	36.5	46.32	35.3	48.08	33.6	50.58	28.5	58.44
Mancozeb	33.3	51.02	31.3	53.97	27.5	59.55	25.3	62.79
Copper oxychloride	50	26.47	46	32.35	42.5	37.5	40	43.23
Carbendazim	24	64.7	20	70.58	18.4	72.94	12.3	82.35
Thirum	34.16	49.76	31.3	53.97	29.5	56.61	27.33	59.8
Propiconazol	0	100	0	100	0	100	0	100
coc + bav.	27.9	58.97	25.33	62.75	21.3	68.67	18.8	72.35
captan + hexa	35.97	47.50	34.26	50	29.5	56.61	27.63	59.63
Control	68.53		68.53		68.53		68.53	
SE(m) ±	**1.12**		**1.065**		**1.265**		**1.173**	
CD at 5 %	**3.328**		**3.165**		**3.758**		**3.501**	

Comment [u3]: Per cent or PPM.....?

Comment [u4]: Per cent or PPM.....?

Comment [u5]: Per cent or PPM.....?

Comment [u6]: Per cent or PPM.....?



Mycelial growth at 10 ppm
% inhibition at 10 ppm
Mycelial growth at 25 ppm
% inhibition at 25 ppm
Mycelial growth at 50 ppm
% inhibition at 50 ppm
Mycelial growth at 100 ppm
% inhibition at 100 ppm
Radial Growth (mm) & growth inhibition(%)
120
100
80
60
40
20
0
Teb..
Hex..
Man..
Cop..
Carb..
Thirum
prop..
coc..
capt..
Cont..
Treatment

Fig 1 : Radial growth and growth inhibition in different treatment efficacy

Conclusions

Based on present investigation it could be concluded that among the Nine chemical fungicides namely Tebuconazole, Hexaconazole, Mencozeb, Copper oxychloride, Carbandazim, Thiram, Propiconazole, Copper oxychloride + Carbandazim and captan + Hexaconazole showed the best result with the maximum inhibition in growth of A. alternata was recorded by Propiconazole (100 %) followed by Tebuconazole (86.02 %) at all the concentrations.

Hence, this finding will be greatly helpful to okra growers by integrating with different management strategies for the purpose of control of okra leaf spot of okra in field condition which may impart positive impact.

Comment [u7]: Is an in vitro study sufficient for okra-growing farmers?

References

Anonymous 2017. Horticulture statistics at a glance 2017. Published by Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, Govt. of India. pp. 458.

Anonymous. 2008. FAOSTAT.

Arain A R, Mithal M, Jiskani, Wagan KH, Khuhro SN and Khaskheli MI. 2012. Report of incidence and chemical control of okra leaf spot disease. Pak. J. Bot. 44(5): 1769-1774.

Moosa, A., S.T. Sahi, I.U. Haq, A. Farzand, S.A. Khan and K. Javaid (2016). Antagonistic potential of Trichoderma isolates and manures against Fusarium wilt of Tomato. International J. Vegetable Sciences 23(3): 207-218.

Peter M, Wiest K, Wiese M, Jacobs, Anne, Morrissey T, Abelson, William W and Michael L. 1987. Reviews of infectious diseases. The university of Chicago press. 9 (4):799-803.

Phapale AD, Solanky, Tayade SC and Sapkale PR. 2010.Investigation on leaf spot [*Alternaria alternata* (Fr.) Keissler.] diseases of okra (*Abelmoschus esculentus* L.) under south Gujrat conditions. M.Sc. (Agri) Thesis submitted to Navsari Agriculture University.

Thippeswami B, Krishnappa M, Chakravarthy CN, Sathisha AM, Jyoti SU and Kumar KV. 2007. Pathogenicity and management of brown lesion and leaf spot in okra caused by *Macrophomina Phaseolina* and *Alternaria alternata*. J Pl. Dis. Sci. 2(1):43-47.

Tohyama AK, Hayashi N, Taniguchi and M Tsuda. 1995. Infection sources and causal agents of *Alternaria* rot of okra pods. Annals of the Phyto-pathological Society of Japan (Japan). 61(4): 346-349.

Sridhar TS and Poonam Sinha. 1989. Assessment of yield losses caused by powdery mildew of okra and its control. Indian J Agric. Sci. 59(9):606-607.

Attri, K., A. Sharma and M. Sharma (2019). Management of Fusarium wilt of bell pepper through fungicides. Journal of Pharmacognosy and Phytochemistry 8(5): 1444-1447.

Bharath, B.G., S. Lokesh, H.S. Prakash and H.S. Shetty (2006). Evaluation of different protectants against seed mycoflora of Watermelon. Research Journal of Botany 1: 1-5.

Patel, M., S. Kumar and S. Mishra (2021). Comparative efficacy of combi fungicides and solo fungicides against Fusarium udum causing wilt of pigeonpea. Pharma Innovation 10(5): 1310-1314.

Pancholi LK, Gupta PK, Gharde Y and Kharte S (2022). *In vitro* assessment of fungicides against *Fusarium oxysporum* f. sp. *pisi* causing Fusarium wilt of pea. Ann. Pl. Protec. Sci. 30 (1) : 18-21

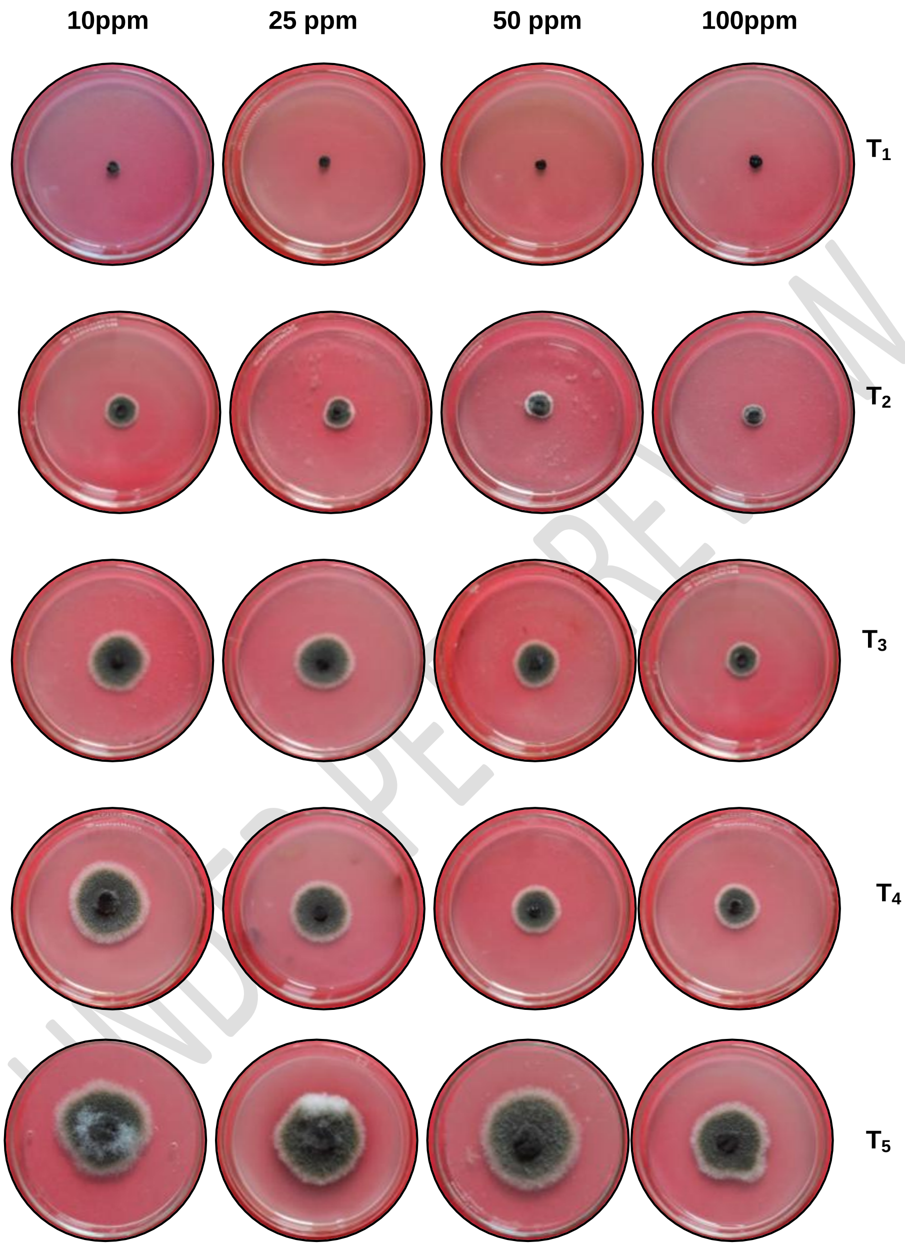


Plate 1 : Effect of Fungicides on mycelial growth of *Alternaria alternata* different concentration after 120 hours

Plate 2 : Effect of Fungicides on mycelial growth of *Alternaria alternata* different concentration after 120 hours

Comment [u8]: How is it possible for all plates to show similar growth with different concentrations of fungicides?

Comment [u9]: : Effect of Fungicides on mycelial growth of *Alternaria alternata* at different concentration after 120 hours