In vitro assessment of fungicides against *Alternariaalternata*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.*Alternariaalternata* 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.

Keywords: Fungicides, chlorotic margins, nematode, Abelmoschusesculentus

1. INTRODUCTION

"Okra,(*Abelmoschusesculentus*(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 (*AlternariaalternataA. chlamydospora*), (Thippeswami*et al*., 2007) powdery mildew (*Erisiphecichoracearum* and *Leveillulataurica*), (Sridhar *et al*.,1989), root / charcoal rot (*Microphominaphaseolina*), wilt (*Fusariumoxysporum* f. sp. *vasinfectum*), Cercospora leaf spot (*Cercosporaabelmoschi, 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, *Alternariaalternata* 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).

*"Alternariaalternata*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*.

2. MATERIAL AND METHODS

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. Theleaf 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.

2.1 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.

2.2 Evaluation of fungicides

In order to find out a suitable fungicide for management ofleaf spot of okra. Nine fungicides, namelyTebuconazole,Propiconazole,Copperoxychloride,Thiram,Carbendazim,Hexaconazole ,Mancozeb,COC+ Carbendazim,Captan + Hexaconazolealong with control was evaluated againstleaf 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)

3. 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 wasTebuconazole (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 of9.5, 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 *Alternariaaltenata*as compared to other fungicides and untreated control after 120hrs of incubation.Inhibitory effect of Propiconazole on mycelia growth of *Alternariaalternata*had been reported by (Phapale*et al.* (2010). “Propiconazolebelong 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 *Fusariumudum”.* (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*conditionsseven fungicides *viz.,*Azoxystrobin, Hexaconazole, Thiram, Tebuconazole, Pyraclostrobin, Carbendazim, Mancozebalong with control was evaluated against *Fusariumoxysporum* 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 *Alternariaalternata*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**	

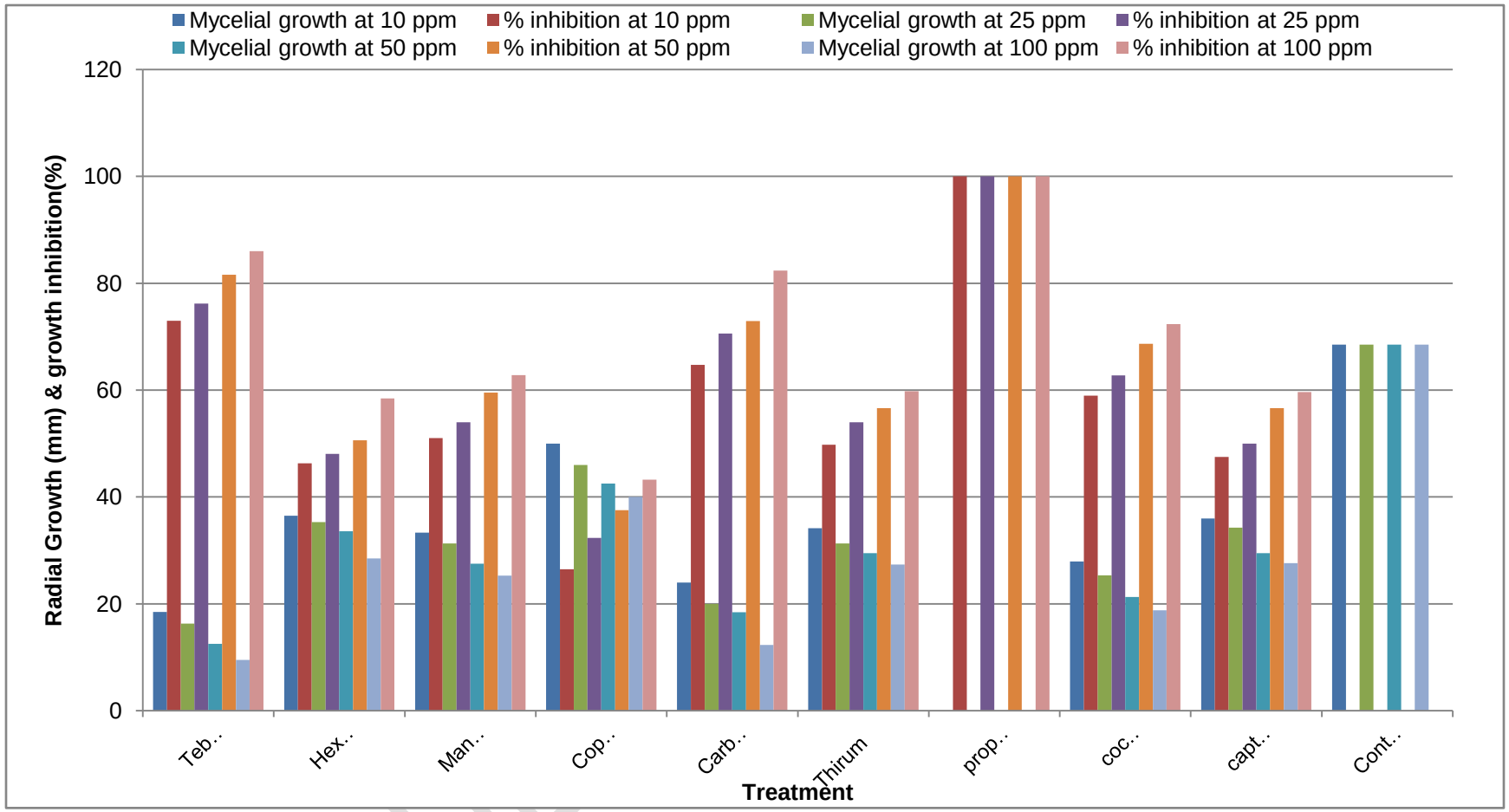
Mycelial growth at 10 ppm
% inhibition at 10 ppm
Mycelial growth at 25 ppm
% inhibition at 25 ppm
Mycelial growth at 50 ppm
% inhibition at 50 ppm
Mycelial growth at 100 ppm
% inhibition at 100 ppm
Radial Growth (mm) & growth inhibition(%)
120
100
80
60
40
20
0
Teb..
Hex..
Man..
Cop..
Carb..
Thirum
prop..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

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

4. 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.

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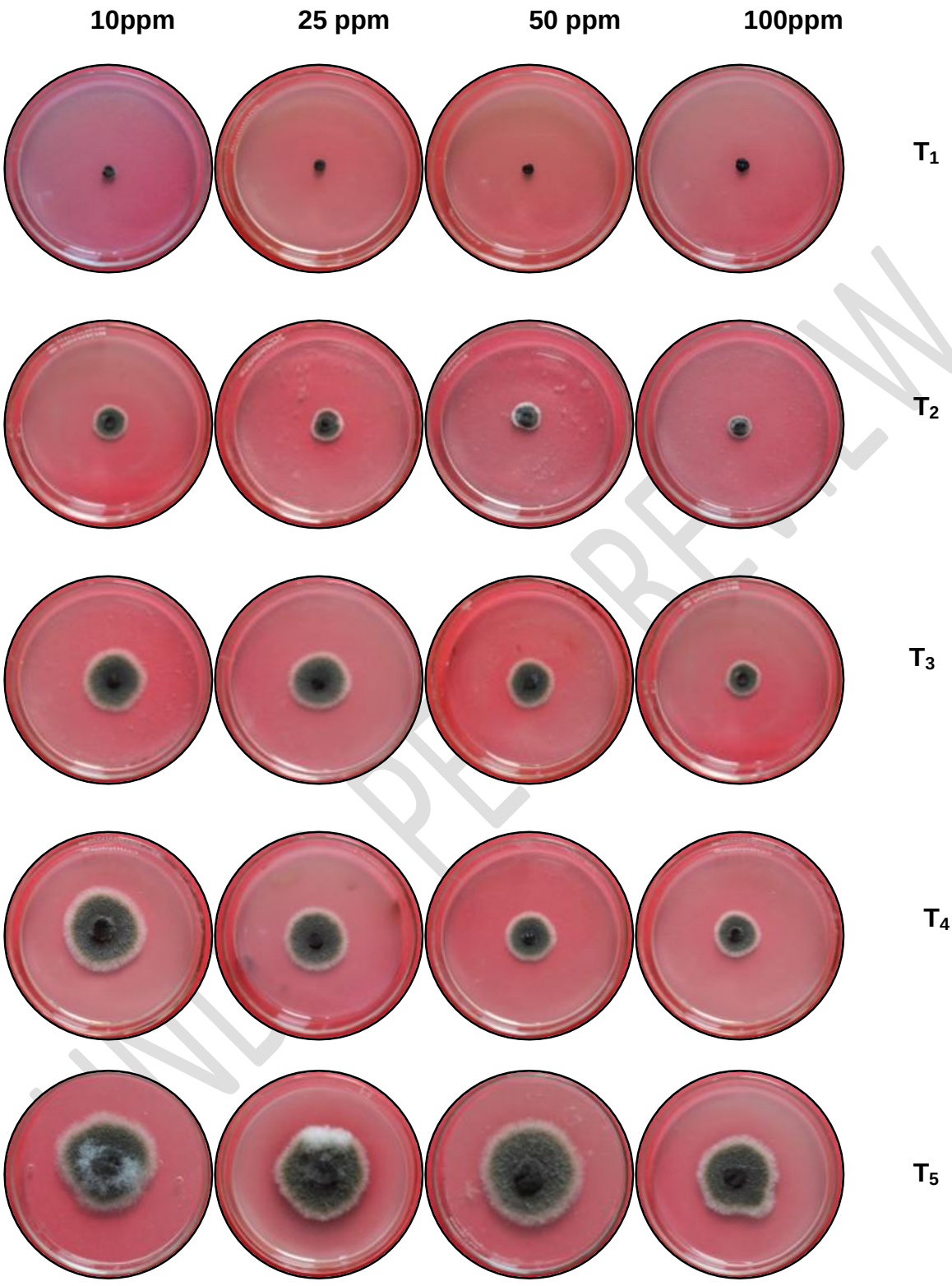

Plate 1 : Effect of Fungicides on mycelial growth of *Alternariaalternata*different concentration after 120 hours

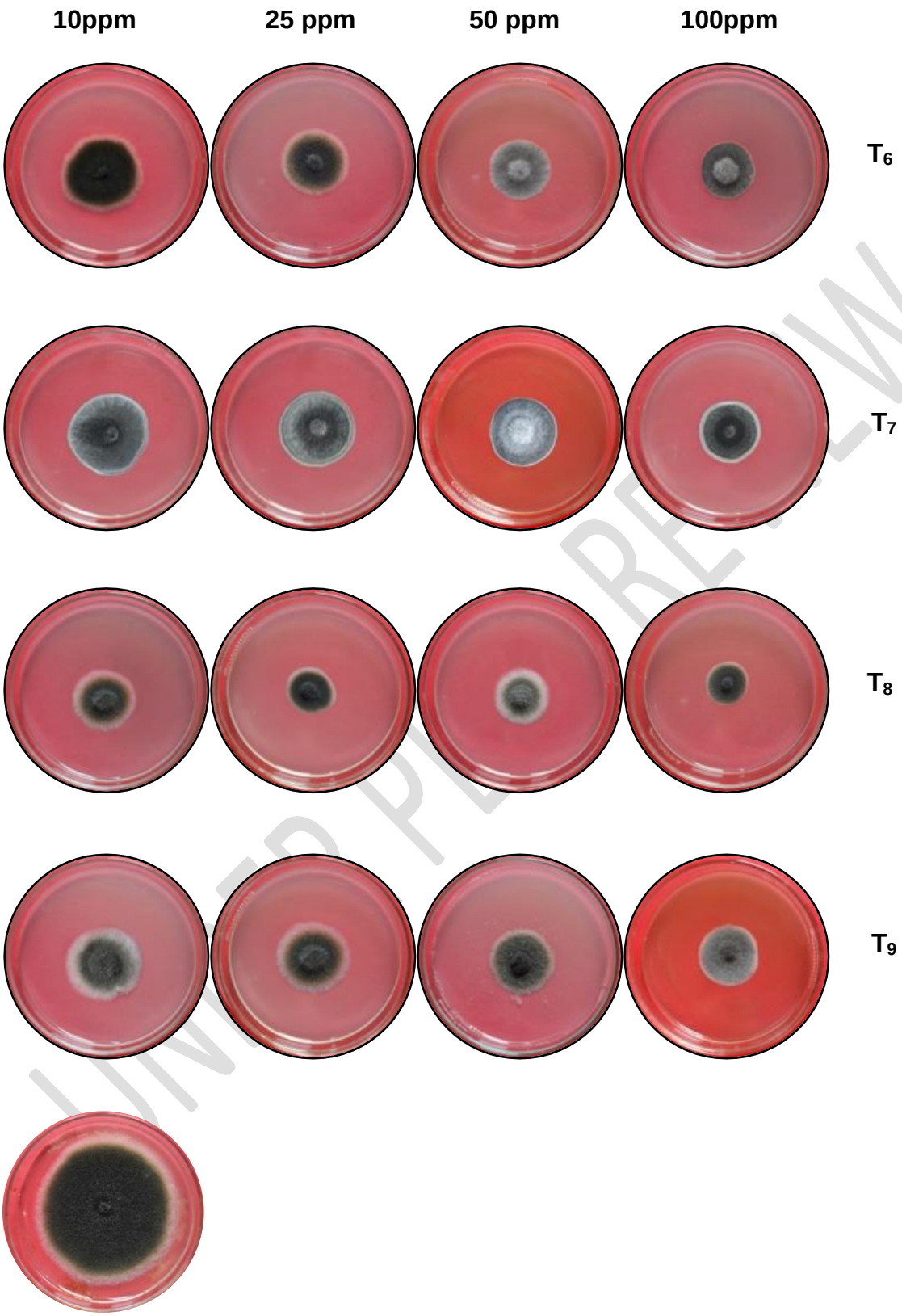

Plate 2 : Effect of Fungicides on mycelial growth of *Alternariaalternate* different concentration after 120 hours