The Andhra Agric. J 69 (2): 244-249, 2022

In vitro Chemical Management of *Stemphylium lycopersici*, inciting Flower Blight Pathogens in Chrysanthemum

A Snehalatharani, V Prasanna Kumari, P. Anil Kumar, K Gopal, K Jayalalitha, V Srinivasa Rao and V S N Deepika

Horticultural Research Station, Kovvur, A. P.

ABSTRACT

Chrysanthemum (*Chrysanthemum indicum* L.) is one of the oldest and widely cultivated flower crops in India. Many pathogens associate with flower blight disease of chrysanthemum including *Phoma ligulicola, Stemphylium* sp., *Alternaria alternata* and *Botrytis cinerea*. In the present study *in vitro* chemical studies were conducted on *Stemphylium lycopersici*, one of the flower blight pathogen of chrysanthemum from Andhra Pradesh. Efficacy of four fungicides *viz.* azoxystrobin, chlorothalonil, difenoconazole and mancozeb was evaluated against *S. lycopersici* at six different concentrations *viz.* 0.01, 0.05, 0.1, 0.15, 0.2 and 0.25 per cent of the fungicides encompassing recommended concentration. We observed that eventhough the chemicals showed differential efficacy against the pathogen, their inhibitory effect is directly proportional to the concentration of the chemical. Mancozeb, irrespective of the dosage used, showed cent per cent inhibition of the pathogen and is found significantly superior to other fungicides tested. Chlorothalonil at recommended concentration of 0.02 % showed 97. 54 % inhibition of the mycelial growth. However, azoxystrobin and difenoconazole at their recommended dose of (0.1%) were significantly inferior and showed 79.05 % and 78.90 % inhibition of the pathogen respectively. The present study provides directions for appropriate selection of fungicides for management of the pathogen under field conditions.

Keywords: *Chrysanthemum, Flower blight, Fungicides*, *Management, Stemphylium lycopersici.*

Chrysanthemum is one of the most important flower crop next only to rose in many countries. As a loose flower and cut flower, the crop fetches valuable returns to the farmers. Crop with its beautiful colours, shapes, arrangement of florets provides an attractive choice for the cultivation by the farmers. However, wide group of pathogens including *Phoma ligulicola, Alternaria* sp., *Stemphylium* sp. and *Botrytis cinerea* affect the crop reducing the quantity and quality of the yield.

Stemphylium species causing ray speck were reported on *Chrysanthemum morifolium*, *Chrysanthemum cinerariaefolium* and *chrysanthemum indicum* (Sarwar and Srinath, 1965, Nishi *et al.,* 2009). The disease was observed frequently on spring and summer grown chrysanthemum plants mainly on fully expanded ray florets. Nishi *et al.* (2009) reported *Stemphylium lycopersici* producing brown or white necrotic specks on ray florets as the causal organism of ray speck of Chrysanthemum from Japan. Symptoms were visible on ray florets, peduncles and involucral bracts.

Stemphylium is a pathogenic genus causing leaf blights, leaf spots, necrotic spots, ray speck and similar diseases in agricultural and horticultural crops throughout the world. Stemphylium blight, once a

minor disease on important crops is now becoming major especially in lentil, tomato, hot pepper and other Solanaceae and Asteraceae crops (Mwakutuya and Banniza, 2010).

From the surveys in chrysanthemum growing areas of Andhra Pradesh, it was observed that ray speck is one of the important factors for flower blight symptoms and there by yield losses. *S. lycopersici* was isolated and characterized morphologically. In the present study, different chemicals were tested for their effectivity against *S. lycopersici* under *in vitro* condition.

MATERIAL AND METHODS

Isolation:

Chrysanthemum flowers infected with blight symptoms, necrosis, flower rot and discoloration of the florets were collected from the surveyed villages during winter 2019 and 2020. Diseased tissue from ray floret was excised, surface sterilized with 70 per cent ethanol and then immersed in 1 % sodium hypochlorite for 2-3 min. The diseased material was then washed thrice with sterile water, blotted dry and were placed on Potato Dextrose Agar (PDA) for incubation at room temperature for 4 days. The resulting cultures were sub cultured on to PDA. Mycelial plugs cut from leading edges of colonies on PDA were stored on PDA slants at 4°C.

***In vitro* efficacy studies**:

Effectiveness of four fungicides at six different concentrations were evaluated against *S. lycopersici* on potato dextrose agar (PDA) under asceptic conditions. The details of fungicides evaluated against the pathogen are given under (Table 1). All the fungicides were tested at six different concentrations, *viz*., 100, 500, 1000, 1500, 2000 and 2500 ppm on active ingredient basis using poisoned food technique. Stock solution of 1,00,000 ppm concentration was prepared using 10 ml of sterilized distilled water. Desired concentration of fungicide was obtained by diluting stock solution using the following formula, $C_1V_1 = C_2V_2$. Where, C_1 = concentration of the stock solution (ppm), V_1 = volume of the stock solution to be added (ml), C_2 = desired concentration (ppm) and V_2 = volume of PDA in which fungicide is to be amended (ml).

Table 1. List of fungicides with their chemical name, trade name and formulation

S. No.	Common Name	Chemical name	Trade Name	Company	Formulation
1	Azoxystrobin	Methyl (E)-2-{2 [6-(2- cyanophenoxy) pyrimidin-4- yloxy]phenyl}-3-methoxyacrylate	Amistar	Syngenta	25% SC
2	Chlorothalonil	2,4,5,6-Tetrachlorobenzene-1,3-dicarbonitrile	Kavach	Syngenta	75% WP
3	Difenoconazole	Difenoconazol 1-((2-(2-Chloro-4-(4-chlorophenoxy) phenyl) -4-methyl-1, 3-dioxolan- 2-yl) methyl)-1H-1,2,4-triazole	Score	Syngenta	25% EC
4	Mancozeb	Manganese zinc ethylene bis dithiocarbomate	Indofil M- 45	Indofil	75% WP

Poisoned medium (20 ml) with respective chemical and concentration was poured in to sterilized petri plate under aseptic conditions. Each plate was inoculated in the centre with five day old fungal culture disc of 5 mm diameter cut from the periphery of actively growing culture and incubated at 28 ± 1 °C in a BOD incubator. PDA plates without chemical served as control. Radial growth of the fungus was recorded till the fungal growth was full in control. Per cent inhibition of growth over control was calculated using the formula given by Vincent (1927).

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

Where,

I = per cent inhibition, C = growth of the fungus in non-poisoned medium and T = growth of the fungus in poisoned food medium.

RESULTS AND DISCUSSION

Efficacy of fungicides on radial growth of *S. lycopersici*

Fungicides, azoxystrobin, chlorothalonil, difenoconazole and mancozeb at six different concentrations, 100 ppm, 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm and 2500 ppm including recommended dose were tested against *S. lycopersici* using poisoned food technique. All the tested chemicals showed efficacy against the pathogen *S. lycopersici* and their inhibitory effect was directly proportional to the concentration of the chemical. Mancozeb, inhibited the pathogen completely and was found significantly superior over other fungicides tested and control.

Even though significantly minimum mycelial growth (0.06 cm) was observed at 2500 ppm concentration, all other concentrations tested were significantly superior (0.06-1.52 cm) over control (6.92 cm) in inhibiting the pathogen even at 8 DAI. Interaction between fungicide and concentration revealed, mancozeb as significantly superior to other fungicides with complete inhibition of the pathogen even at 100 ppm. The inhibitory effect of mancozeb was significantly superior over other fungicides tested at different concentrations was on par only at higher concentration with the tested fungicides.

All the tested fungicides were effective against *S. lycopersici* with cent per cent reduction of mycelial growth by mancozeb when compared to control. Chlorothalonil at recommended concentration of 2000 ppm showed 97. 54 % reduction in the mycelial growth. However, the other fungicides, azoxystrobin

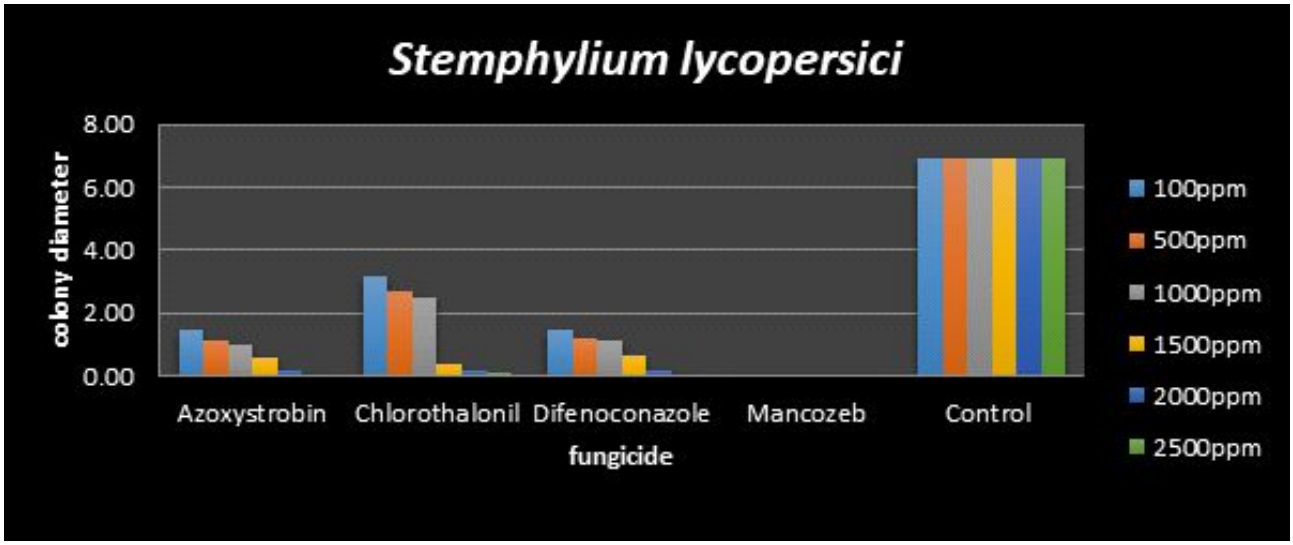

Fig 1. Graph depicting *in vitro* efficacy of fungicides against *S. lycopersici*

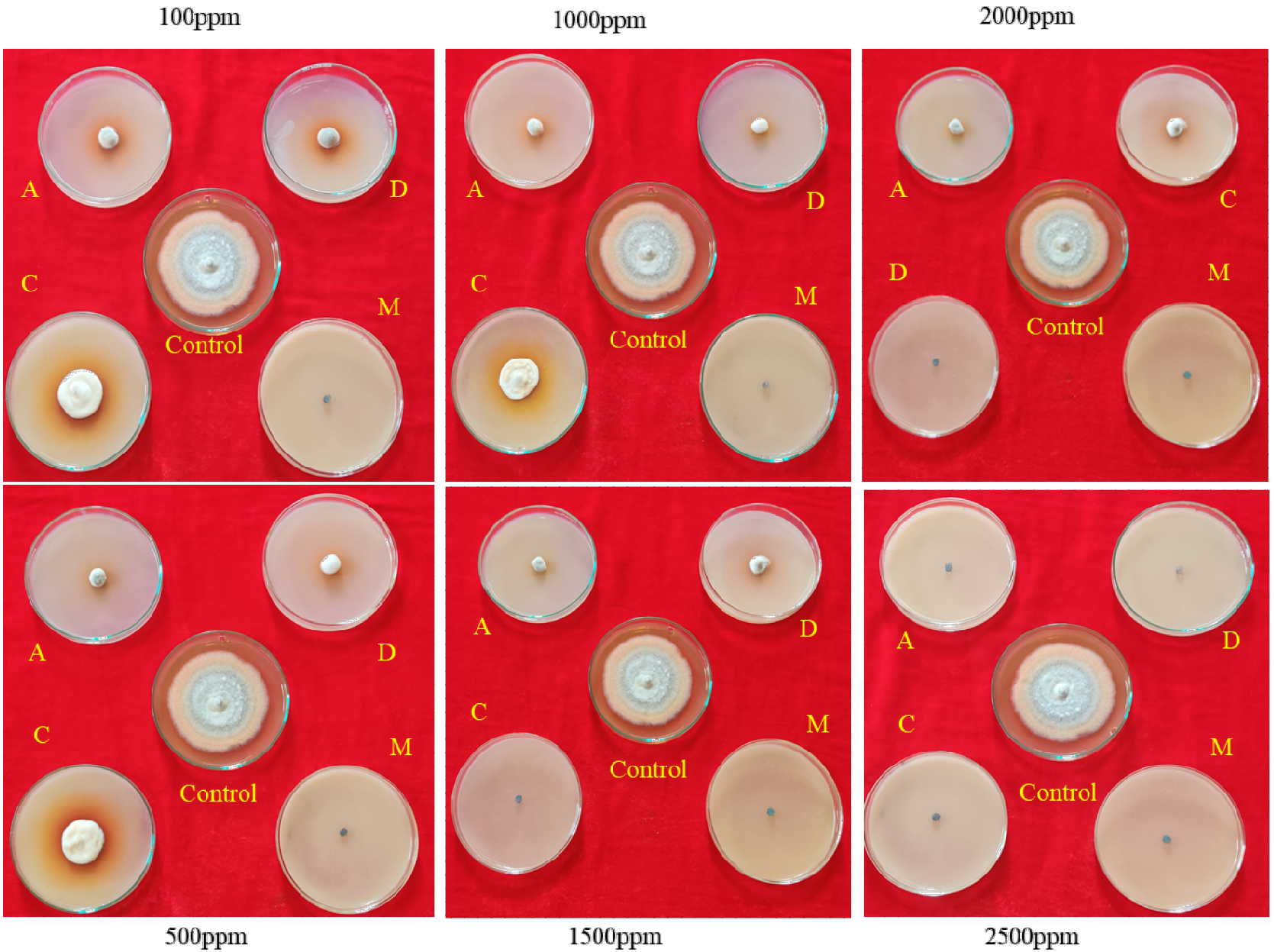

Plate 1. ***In vitro*** **efficacy of fungicides against** ***Stemphylium lycoperici*****: A: Azoxystrobin; C: Chlorothalonil; D: Difenoconazole; M: Mancozeb**

Table 2. Effect of fungicides on radial growth of *S. lycopersici in vitro*

Fungicides/ concentration	Colony diameter (cm)							Per cent inhibition						
	100 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm	2500 ppm	Mean	100 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm	2500 ppm	Mean
Azoxystrobin	1.45 (1.57)	1.14 (1.46)	0.99 (1.41)	0.6 (1.26)	0.2 (1.10)	0.07 (1.03)	0.74 (1.31)	79.05	83.53	85.69	91.33	97.11	98.99	89.28
Chlorothalonil	3.16 (2.04)	2.7 (1.92)	2.49 (1.87)	0.37 (1.17)	0.17 (1.08)	0.1 (1.05)	1.5 (1.52)	54.34	60.98	64.02	94.65	97.54	98.55	78.35
Difenoconazole	1.46 (1.57)	1.18 (1.48)	1.11 (1.45)	0.63 (1.28)	0.2 (1.10)	0.07 (1.03)	0.78 (1.32)	78.9	82.95	83.96	90.9	97.11	98.99	88.8
Mancozeb	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean	1.52 (1.59)	1.26 (1.50)	1.15 (1.47)	0.4 (1.18)	0.14 (1.07)	0.06 (1.03)		-	-	-	-	-	-	-
Control	6.92 (2.81)							78.07	81.87	83.42	94.22	97.94	99.13	-

Mean of three replicates Figures in parenthesis are arc sine transformed values

and difenoconazole at their recommended dose (1000 ppm) were inferior to the other two fungicides and showed 79.05 % and 78.90 % inhibition of the pathogen respectively. Azoxystrobin, difenoconazole and chlorothalonil at 2500 ppm concentration resulted in 98.99, 98.99 and 98.55 per cent reduction over control respectively (Table 2, Fig 1, Plate 1).

Present findings are in accordance with Rahman *et al.* (2010) who reported cent per cent inhibition of mycelial growth of lentil *S. botryosum* using mancozeb (500, 1000, 1500 and 2000 ppm concentrations). Significantly high mean per cent inhibition (90.01) of *S. vesicarium* causing leaf blight of onion mycelial growth by mancozeb was reported by Harde *et al.* (2014).

CONCLUSION

Identification of causal organism and *in vitro* fungicide efficacy studies provides earlier knowledge for management of the disease under field conditions. The effective fungicides found from the above study could be exploited for the management of chrysanthemum flower blight disease under field conditions.

LITERATURE CITED

Harde A L Suryawanshi A P and Lambe K M 2014 Evaluation of fungicides (*in vitro*) against *Stemphylium vesicarium*, causing leaf blight of Onion. *Trends in Biosciences*.7 (17): 2425-2429.

Mwakutuya E and Banniza S 2010 Influence of temperature and wetness periods on the development of Stemphylium blight on lentil. *Plant Disease,* 94:1219-1224.

Nishi N Muta T Ito Y Nakamura M and Tsukiboshi T 2009 Ray speck of chrysanthemum caused by *Stemphylium lycopersici* in Japan. *Journal of General Plant Pathology,* 75:80–82

Rahman T Ahmed A U Islam M R and Hosen M I 2010 Physiological Study and both *in vitro* and *in vivo* Antifungal Activities against *Stemphylium botryosum* causing Stemphylium Blight Disease in Lentil (*Lens culinaris*). *Plant Pathology Journal,* 9: 179-187.

Sarwar M and Srinath K V 1965 A new species of *Stemphylium* on *Chrysanthemum cinerariaefolium. Central Indian Medicinal Plants Organization.* 21 IV. India.

Vincent J M 1927 Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 59: 850.