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Studies on *Alternaria* leaf spot disease of china aster and evaluation of fungicides under *in vitro* conditions

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Abstract

The genus *Callistephus*, meaning “a crown,” belongs to the family Asteraceae. The present study was undertaken to investigate the symptomatology, isolation, morphological characteristics, and *in vitro* chemical management of *Alternaria* leaf spot. The disease symptoms were characterized by the appearance of small (1–2 mm), dark to light brown, circular to irregular lesions on leaves, often exhibiting distinct concentric rings. Infected plant tissues were collected and used for the isolation of pathogen. using standard tissue isolation techniques. The colony initially appeared light grey and gradually turned dark grey to olivaceous with age, exhibiting fast and uniform growth that covered the entire Petri plate. The spores (conidia) were dark brown to black, multicellular, and muriform, possessing both transverse and longitudinal septa. The *In vitro* evaluation of fungicides against mycelial growth revealed significant differences among treatments at concentrations of 25, 50, and 100 ppm. Among all fungicides tested, Prochloraz 24.4% + Tebuconazole 12.1% showed complete inhibition (100%) of mycelial growth at all concentrations, indicating superior efficacy. Other combination fungicides, including Tebuconazole 50% + Trifloxystrobin 25% w/w, Captan 70% + Hexaconazole 5% WP, and Flusilazole 12.5% + Carbendazim 25% SE, also achieved 100 per cent inhibition at higher concentrations (50 and/or 100 ppm). whereas lower inhibition was recorded with Carbendazim + Mancozeb WP (54.07%) and Mancozeb 75 WP (47.41%). A gradual increase in inhibition percentage was observed with increasing fungicide concentration. Overall, combination fungicides, particularly those comprising triazole and strobilurin groups, were found to be more effective than contact fungicides in inhibiting the mycelial growth of *Alternaria* sp.

Keywords: *Alternaria* leaf spot, *in vitro*, fungicide and evaluation

Introduction

The genus *Callistephus*, meaning “a crown” in English, belongs to the family Asteraceae, which also includes chrysanthemum, gerbera, dahlia, marigold, zinnia, and coreopsis. It is a monotypic genus containing a single species, *Callistephus chinensis* (L.) Nees, commonly known as China aster or annual aster. This species is native to China and Korea (Desai, 1967) [3].

China aster is a highly attractive annual flowering plant known for its vibrant colours and profuse blooming. It is widely cultivated both in open fields and under protected conditions for cut and loose flowers. In addition, it is popular as a potted plant and is extensively used for garden decoration (Hay *et al.*, 1976) [4]. Among traditional flowers, China aster ranks third in popularity, alongside chrysanthemum and marigold.

Commercial cultivation is practiced in several countries, including India, France, the Netherlands, the United Kingdom, Russia, North America, Japan, and Switzerland. In India, it is predominantly grown in Karnataka, Tamil Nadu, Telangana, Andhra Pradesh, Maharashtra, and West Bengal, covering an area of approximately 3,500 hectares (Kumari *et al.*, 2017) [7]. Karnataka alone accounts for about 207 hectares, with a production of 1,448 metric tonnes and a productivity of 7.01 metric tonnes per hectare (Anonymous, 2020) [1]. The Bengaluru region, in particular, is well suited for year-round cultivation due to its favourable climate and strong market demand.

To ensure continuous production and supply, China asters are cultivated throughout the kharif, rabi, and summer seasons under open field conditions. However, production is significantly

affected by several biotic stresses, among which leaf spot disease caused by *Alternaria alternata* (Fr.) Keissler is a major constraint. This disease can result in considerable yield losses across growing regions (Nagrle *et al.*, 2012) ^[9].

Due to the limited availability of disease-resistant China aster cultivars, farmers largely rely on fungicides for disease management. However, the indiscriminate use of these chemicals can lead to the accumulation of harmful residues in soil, water, and plant systems. Hence, identifying effective and judicious fungicide options is essential for sustainable disease control. In this context, the present study evaluates the *in vitro* efficacy of fungicides against *Alternaria alternata*, the pathogen causing leaf spot in chain aster is under taken.

Materials and Methods

Isolation of the Pathogen

Infected plant leaves collected and cut into small pieces using a sterile blade, ensuring that each segment contained a portion of healthy tissue. The tissue pieces were surface sterilized in 0.1% sodium hypochlorite (NaOCl) solution for 50–60 seconds, followed by three successive rinses with sterile distilled water in Petri plates. The sterilized tissues were then placed on sterile blotting paper to remove excess moisture. After drying, the tissue segments were aseptically transferred onto 90 mm Petri plates containing potato dextrose agar (PDA) medium and incubated under laboratory conditions.

Purification of the Pathogen

Pure cultures of the pathogen were obtained using the single spore isolation technique. A spore suspension was prepared from a sporulated culture and serially diluted up to 10^{-6} . One millilitre of the diluted suspension was dispensed into sterilized Petri plates, followed by the addition of 1.5% sterilized water agar. The plates were incubated at room temperature for approximately 8 hours. The plates were then observed under a stereo binocular or compound microscope to identify individual germinated spores. These were marked and aseptically transferred to fresh PDA plates to obtain pure cultures.

In vitro Evaluation of Fungicides Against the Pathogen

The efficacy of 14 fungicides, including contact, systemic, and combination types, was evaluated against *Alternaria Sp.* using the poisoned food technique under laboratory conditions. The required quantity of each fungicide was calculated based on its formulation and incorporated into molten PDA medium to obtain final concentrations of 25, 50, and 100 ppm. Approximately 20 ml of fungicide-amended medium was poured into sterile Petri plates and allowed to solidify.

A 5 mm diameter mycelial disc from an actively growing culture of the pathogen was placed at the center of each plate. Plates without fungicide served as the control. All treatments were replicated, and plates were incubated under suitable conditions. Radial growth of the fungus was recorded, and the per cent inhibition of mycelial growth was calculated using the formula given by Vincent (1947) ^[11]:

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where:

C = Growth of the pathogen in control plates

T = Growth of the pathogen in treated plates

Results and Discussion

Isolation and purification of pathogen

The symptoms of *Alternaria* leaf spot are characterized by the appearance of small (1–2 mm), dark to light brown, circular to irregular lesions on leaves, often exhibiting distinct concentric rings. Infected plant tissues were collected and used for the isolation of *Alternaria* sp. following the tissue segment method. The pathogen was successfully isolated on Potato Dextrose Agar (PDA) medium, where it exhibited good mycelial growth. Sporulation was observed 9–10 days after incubation, and the culture was further purified through single spore isolation as described in the Materials and Methodssimilar finding ere also observed by Mallikarjunaiah, R.R. and Rao, V.G., (1972) ^[8].

Cultural and Spore Morphological Characteristics

The fungal colony on Potato Dextrose Agar (PDA) initially appears whitish to light grey and gradually turns dark grey to olivaceous with age. The colony is fast-growing, spreading uniformly to cover the entire Petri plate. The surface is cottony to woolly with a dense, fluffy mycelial mat. Concentric zonation's are often visible, giving a characteristic ring-like appearance.

The center of the colony appears slightly raised and darker due to abundant sporulation, while the peripheral region remains lighter and actively growing. The reverse side of the colony typically shows dark brown to black pigmentation. Sporulation is profuse after 7–10 days of incubation (fig 01).

The spores (conidia) of *Alternaria* sp. are typically dark brown to black in color and multicellular, possessing both transverse and longitudinal septa (muriform). They are usually obclavate to ellipsoidal in shape, often with a tapering beak at the apex (fig 01). The sililar results were obtained by Nagrle, D.T *et al* (2013) ^[10] in gerbera *Alternaria* leaf spot.

In vitro evaluation of different fungicides against

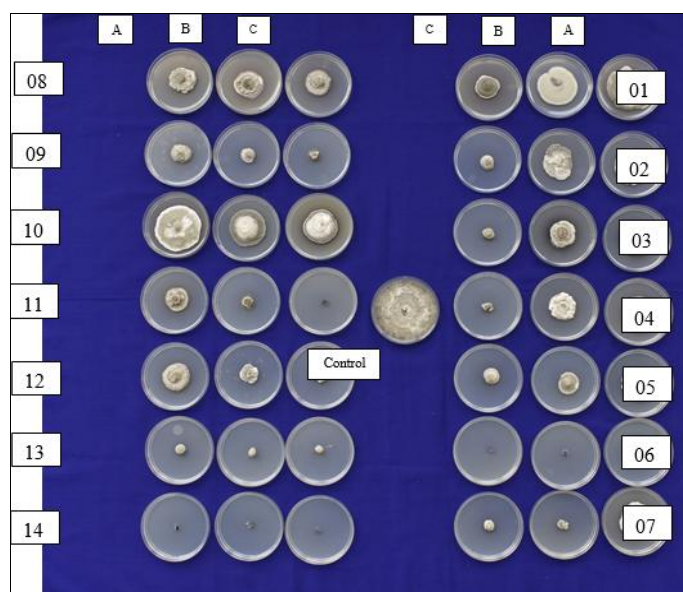
The *In vitro* evaluation of fungicides against mycelial growth of pathogen revealed significant differences among treatments at varying concentrations (25, 50, and 100 ppm). Among all the fungicides tested, Prochloraz 24.4% + Tebuconazole 12.1% exhibited complete inhibition (100%) of mycelial growth at all concentrations, indicating its superior efficacy. Similarly, combination fungicides such as Tebuconazole 50% + Trifloxystrobin 25% w/w, Captan 70% + Hexaconazole 5% WP, and Flusilazole 12.5% + Carbendazim 25% SE also recorded 100 per cent inhibition at higher concentrations (50 and/or 100 ppm).

Table 1: *In vitro* evaluation of fungicides against *Alternaria* sp. of China aster

Sl. No.	Fungicides	Per cent inhibition of mycelia growth		
		25 PPM	50 PPM	100 PPM
1	Mancozeb 75 WP	34.44(35.94)	59.26(50.39)	47.41(43.51)
2	Tebuconazole 25 EC	45.56(42.45)	57.78(49.48)	78.89(62.65)
3	Propiconazole 25EC	48.15(43.94)	60.00(50.77)	86.30(68.28)
4	Hexaconazole 5 SC	55.93(48.41)	61.11(51.42)	88.89(70.53)
5	Difenconazole 25 EC	60.00(50.77)	58.52(49.91)	71.85(57.99)
6	Tebuconazole 50%+ Trifloxystrobin 25% w/w	75.93(60.65)	100.00(89.75)	100.00(89.75)
7	Propiconazole13.9% + Difenconazole 13.9% EC	46.30 (42.88)	76.30(60.88)	87.78(69.55)
8	Zineb 68 + Hexaconazole 4 WP	57.41(49.26)	62.22(52.08)	64.81(53.62)
9	Captan 70 + Hexaconazole 5 WP	66.67(54.74)	84.44(66.78)	100.00(89.75)
10	Carbendazim 12+ Mancozeb 63 WP	35.19 (36.36)	45.93(42.66)	54.07 (47.34)
11	Flusilazole 12.5% + Carbendazim 25% SE	60.00(50.77)	83.33(65.91)	100.00(89.75)
12	Azoxystrobin 11 + Tebuconazole 18.3 SC	61.11(51.42)	71.11(57.49)	82.22(65.07)
13	Fluxapyroxad 250 g/L + Pyraclostrobin 250 g/L SC	80.00(63.44)	85.56(67.67)	90.00(71.58)
14	Prochloraz 24.4% + Tebuconazole 12.1%	100.00(89.75)	100.00(89.75)	100.00(89.75)
	Fungicides	Concentration	F×C	
	S. Em ±	0.49	0.21	0.96
	CD (P=0.01)	1.64	0.76	2.84

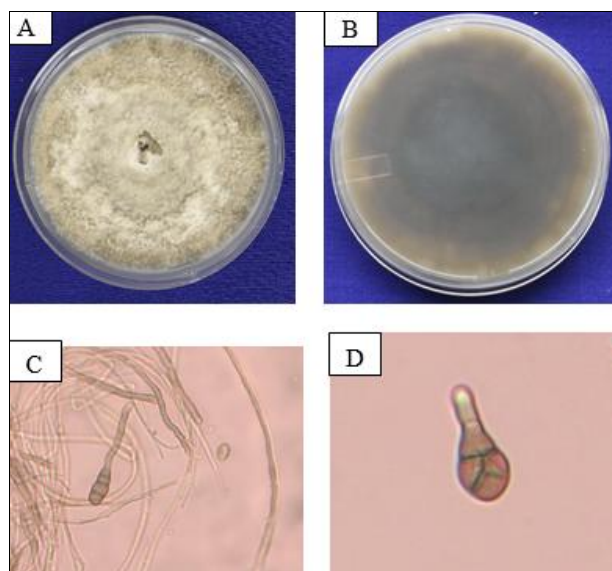
At 100 ppm concentration, Hexaconazole 5 SC (88.89%), Propiconazole 25 EC (86.30%), and Propiconazole 13.9% + Difenconazole 13.9% EC (87.78%) showed high levels of inhibition. Which were agreement with Banne *et al.* (2021) [2] and Kumar *et al.* (2011) [6] where they found Propiconazole 25% EC and Hexaconazole 5% EC showing 100% inhibition. Which was followed by Fluxapyroxad + Pyraclostrobin SC (90.00%) and Azoxystrobin + Tebuconazole SC (82.22%). Moderate inhibition was observed in Difenconazole 25 EC (71.85%) and Zineb + Hexaconazole WP (64.81%) Strobilurins inhibits mitochondrial respiration and Hexaconazole and Tebuconazole target sterol biosynthesis, collectively providing robust control over *Alternaria* sp, whereas comparatively lower inhibition was recorded with Carbendazim + Mancozeb WP (54.07%) and Mancozeb 75 WP (47.41%) (Table. 01, fig. 02).

At lower concentrations (25 and 50 ppm), a gradual increase in inhibition percentage was observed with increasing concentration for most fungicides. Overall, combination fungicides, particularly those containing triazole and strobilurin groups, were found to be more effective in inhibiting mycelial growth compared to contact fungicides



A.25 ppm B.50 ppm C.100 ppm

1.Mancozeb 75 WP 2. Tebuconazole 25 EC 3. Propiconazole 25EC 4. Hexaconazole 5 SC 5. Difenconazole 25 EC 6. Tebuconazole 50%+ Trifloxystrobin 25% w/w 7. Propiconazole13.9% + Difenconazole 13.9% EC 8. Zineb 68 + Hexaconazole 4 WP 9.Captan 70 + Hexaconazole 5 WP 10. Carbendazim 12+ Mancozeb 63 WP 11. Flusilazole 12.5% + Carbendazim 25% SE 12. Azoxystrobin 11 + Tebuconazole 18.3 SC 13. Fluxapyroxad 250 g/L + Pyraclostrobin 250 g/L SC 14. Prochloraz 24.4% + Tebuconazole 12.1%

Fig 2: *In vitro* evaluation of fungicides against *Alternaria* sp. of China aster

A. Front view. C. Septate hyphae and transverse septate spore.
B. Backside view. D. Transverse and longitudinal septate spore.

Fig 1: Colony and spore morphological characters of *Alternaria* sp.

Conclusion

The present study clearly demonstrates that combination fungicides are highly effective in inhibiting the mycelial growth of *Alternaria* spp. under *in vitro* conditions. Among the tested treatments, Prochloraz 24.4% + Tebuconazole 12.1% showed complete (100%) inhibition at all concentrations, indicating its superior efficacy. Other combination fungicides such as Tebuconazole 50% + Trifloxystrobin 25% w/w, Captan 70% + Hexaconazole 5% WP, and Flusilazole 12.5% + Carbendazim 25% SE also exhibited complete inhibition at higher concentrations (50 and/or 100 ppm). The enhanced effectiveness of these fungicides can be attributed to their multiple modes of action.

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