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Survey, characterization and management of leaf blight of Chrysanthemum caused by *Alternaria alternata* (Fries) Keissler

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ABSTRACT: Leaf blight caused by *Alternaria alternata* is one of the most important foliar diseases hindering the cultivation of chrysanthemum as it infects both leaves and flowers. The survey conducted in eastern dry zone of Karnataka, India revealed highest disease severity at Chikkanahalli in Tumakuru district and the least disease severity (8%) at Mavahalli in Kolar district. The pathogen produced conidia which are typically muriform, dark brown, thick walled. The conidium was up to 33.93 – 57.42 µm long and 10.44 – 18.27 µm wide with 6 - 7 transverse septa and 1 - 3 longitudinal septa. (Majority of conidia are non-beaked few with short rudimentary dark brown beaks, with a range of 13.05 - 26.10 µm length). Molecular confirmation of the causal organism was done through PCR amplification and sequencing of ITS region. *In-vitro* evaluation of fungicides revealed, among contact, systemic and combi-products tested copper oxy chloride 50%WP, hexaconazole 5% SC and Zineb 68% + hexaconazole 4% WP and tricyclazole 18% + mancozeb 62% WP respectively were effectively inhibited the pathogen. *In-vivo* evaluation of fungicides revealed, foliar application of copper oxy chloride 50% WP @ 0.3 per cent proved to be highly effective in arresting spread of the disease.

Keywords: Chrysanthemum, *Alternaria alternata*, leaf blight, ITS (Internal transcribed Spacer), characterization

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora* Ramat.) is a multi-use flower crop belonging to Asteraceae family and gaining more popularity as a cut flower, loose flowers and pot plant. Chrysanthemum is commonly known as Queen of East produces very attractive flowers of different shape, size and colours. It is an important commercial flower next to rose in the international florist's trade and grown throughout the world (Kher, 1990). In Karnataka, it is being cultivated extensively in Bengaluru Urban, Bengaluru Rural, Mysore, Tumkur, Kolar, Chikkaballapur, Dharwad and Belgaum districts with an area of 4978 ha with 6006 metric tonnes (MT) of production and with an average productivity of 11.60 metric tonnes per hectare (Anon., 2015).

Chrysanthemum flowers are mainly used as loose flowers in garland making and cut flowers for bouquet making. The crop is cultivated both in open field and green houses. Chrysanthemum is prone to infection by several pathogens including fungi, bacteria, virus, viroid and nematode, which cause damage to roots, stem, leaves and flowers. The different fungal diseases are leaf spot (*Alternaria alternata*), white rust (*Puccinia horiana*), Dry rot/ Crown rot (*Rhizoctoni solani*), wilt (*Verticilium* spp., *Fusarium oxysporum*), root rot (*Pythium* spp., *Phytophthora* spp.), leaf spot (*Septoria chrysanthemella*), gray mould (*Botrytis cineria*), black rot (*Ascochyta* spp.), stem rot (*Fusarium solani*), powdery mildew (*Spacelotheca* spp.). Bacterial diseases *viz*., bacterial leaf spot (*Pseudomonas cichori*), crown galls tumors (*Agrobacterium tumefaciens*) and viral diseases like mosaic and stunt (Pradeep kumar *et al*., 2008) are the few common diseases.

Among these diseases, the chrysanthemum blight caused by *Alternaria alternata* (Fries.) Keissler has been found to be the most important disease, adversely affecting quality and yield loss upto 80 to 90 per cent in field as well as in market conditions (Kumar, 2008). Since there are no sources of resistance available for the disease management, farmers are largely depended on use of fungicides to manage this disease. Looking in to these bottle necks and also to tackle the problem of fungicidal resistance the present investigation was undertaken to identify the new effective fungicides, which derive maximum benefit to the farmers.

MATERIALS AND METHODS

Survey for leaf blight of chrysanthemum in major growing areas in the eastern dry zone of Karnataka

Roving survey was conducted in the chrysanthemum growing areas of Kolar, Chikkaballapur, Tumkur and Bengaluru Rural districts of Karnataka during

September, 2018 to January, 2019. For field survey, the places were selected spreading across different districts mentioned (Table 1). During survey the disease severity was estimated by recording symptom severity using 0-5 scale (Mridha *et al*., 2007; Ghosh *et al*., 2009) in plants in the field, where ten plants were randomly selected in $10m^2$ area and five such areas were selected for one acre crop. Where, 0 - No disease symptoms, 1 - A few spots towards tip covering 10 per cent leaf area, 2 - Several dark brown patches covering upto 20 per cent leaf area, 3 - Several patches with paler outer zone covering upto 40 per cent leaf area, 4 - Leaf blight covering upto 75 per cent leaf area or breaking of the leaves from centre, 5 - Complete drying of the leaves or breaking of the leaves from centre.

The recorded grade values were converted into Per cent Disease Index (PDI) by using following formula proposed by Wheeler (1969).

$$PDI = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings x maximum disease grade recorded}} \times 100$$

During survey, the information on the following aspects was recorded namely, District, taluk, village, farmer's name, crop stage, name of the cultivar, and soil type, open or polyhouse conditions, the other pests and diseases noticed and plant protection measures followed.

Isolation of the pathogen

The samples brought from the field, showing typical symptoms of leaf blight disease were subjected for dissection and microscopic observation and the pathogens were identified morphologically. The pathogen that occured repeatedly in higher number of fields surveyed was tried to cultured on PDA medium. The standard tissue isolation procedure was followed to isolate the pathogen. The infected leaf bits were surface sterilized with 1:1000 sodium hypochlorite solution for 30 seconds and repeatedly washed separately in sterilized distilled water to remove the traces of chemical solution if any and then transferred to sterilized petri plates (1-2 leaf bits per Petri dish) containing potato dextrose agar (PDA).

The petri plates were incubated at room temperature (27±1°C) and observed periodically for the growth of the fungus. Bit of fungal growth developed from the infected tissue was transferred to PDA slants and incubated at 27°±1°C for 12 days. Then such slants with pure culture were used for further studies.

Identification and characterization of the pathogen

Identification of the fungus was made after examining about one hundred conidia under microscope (under 10x) from mature pure culture of the fungus obtained from infected leaves of chrysanthemum. To study the morphological characters, stage and ocular micrometry were used to measure the length and breadth of conidium and beak length and septal number. These observations were compared with those of the standard measurements given by Ellis (1971) to identify the pathogen.

Molecular characterization

Isolation of total genomic DNA and amplification

Total DNA was isolated from seven days old pathogen culture grown on potato dextrose broth under continuous agitation at 26 °C using Cetyl-Trimethyl Ammonium Bromide (CTAB) method (Kajal *et al*., 2018). Amplification was done using ITS_1 (5'- TCCGTAGGTGAACCTGCGG -3') and ITS_4 (5'- TCCTCCGCTTATTGATATGC -3') primers with denaturation at 94°C for 6 min., annealing temperature of 55°C for 1 min. and final extension at 72°C for 30 min. The amplified PCR product was cut from the gel and purified using minicolumn, purification resin and buffer, according to the manufacturer's protocols (High Pure PCR Product Purification Kit; Roche Mannheim, Germany). The purified amplicon was sent for sequencing along with details (host name, primers used and quantity of product).

Phylogenitic analysis

The sequences so obtained were used for NCBI – Blast analysis and using Bioedit, ClustalW and phylogenetic relationship of *A.alternata* was established. The dendrogram was constructed using MEGA–X (https;// www.megasofware.net) after alignment of sequences through Clustal - W.

***In vitro* evaluation of fungicides**

The efficacy of contact, systemic and combi product fungicides against blight pathogen were assessed by poisoned food technique. Required quantities of individual fungicides were added separately into molten and cooled potato dextrose agar so as to get the desired concentration of the fungicides. Later 20 ml of the poisoned medium was poured into sterile petri plates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut out by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicides to the medium. Each treatment of contact fungicide and

combi products was replicated thrice while the systemic fungicides with five replications. Then such plates were incubated at room temperature for eight days and radial colony growth was measured. The efficacy of a fungicide was expressed as radial growth of mycelium over control and per cent inhibition of mycelial growth over control was calculated by using the formula given by Mridha *et al.* (2007). The growth values were subjected to square root transformations and the data were analyzed statistically.

***In vivo* evaluation of fungicides**

The field experiment was laid out during *kharif / rabi* 2018-19 in Randomized Block Design (RBD) with three replications under natural conditions. Totally three sprays were given at 15 days interval, starting from the initiation of the disease. The observations on leaf blight disease were recorded before each spray, where six plants were selected and labeled from each treatment and a 0-5 scale (Mridha *et al*., 2007; Ghosh *et al*., 2009) was used for recording the disease severity of leaf blight (Table1). The Per cent disease index (PDI) was calculated by using the formula of Wheeler (1969).

RESULTS AND DISCUSSION

Distribution of the disease: The roving survey carried out during *rabi* 2018-19 in four major chrysanthemum growing districts in Eastern dry zone of Karnataka *viz*., Kolar, Chikkaballapur, Tumakuru and Bengaluru rural revealed that the *Alternaria* leaf blight disease was severe in all the surveyed districts and disease severity ranged from 8.00 to 86.00 per cent. The highest severity (86%) of *Alternaria* leaf blight was noticed in the fields of Chikkanahalli village in Tumakuru district, whereas least disease severity (8%) was recorded at Mavahalli village in Kolar district (Table 1). The highest district average of the per cent disease index (PDI) was recorded in Tumakuru (45.66%) followed by Kolar (42.66%), Chikkaballapur (40.33%) and the least PDI was recorded at Bengaluru rural (38%). The variation in the disease severity in different districts may be due to variation in cultural practices, distribution of the rainfall and plant protection measures followed. Kolte, (1984) reported that higher rainfall and relative humidity was reported to cause severe epidemics of *Alternaria* blight of sunflower. Continuous cultivation of any crop over the season and years build up inoculum level to such an extent that the epidemics become a common phenomenon (Kumar, 2008).

The symptoms of *Alternaria alternata* observed in all growing areas infecting on aerial parts of the plant and produced symptoms on foliar parts as minute brown circular spots, which enlarge at later stages of infection (Plate 1).

Morphological features of *Alternaria*

In culture, the fungal growth was initially white, cottony with profuse aerial mycelium which gradually turned greenish brown. Aged culture appeared completely black with no aerial mycelium. Conidia were observed to measuring about 33.93 – 57.42 µm long and 10.44 – 18.27 µm wide. Conidia are typically muriform, dark brown, thick walled. Majority of the conidia were non-beaked few with short rudimentary dark brown beaks, with a range of 13.05 - 26.10 µm length, conidia had 6 - 7 transverse septa and 1 - 3 longitudinal septa (Table 2 and Plate 2). Based on the characters of the colony

Symptoms on leaves

Symptoms on flowers

Plate 1: Symptoms of blight caused by *Alternaria alternata* in chrysanthemum

Table 1. Per cent disease index of leaf blight of chrysanthemum in different parts of Eastern dry zone of Karnataka during *rabi* 2018-19

District	Taluk	Village	Variety grown	Stage of crop (DAP)	Per cent disease index (PDI)		Other disease
					Alternata leaf blight	Septoria leaf blight	
Kolar	Bangarpet	Nayakarahalli	Poornima white	115	74.00		-
		Madamangala	Poornima white	80	50.00	36.00	White rust
		Nernahalli	Green valley marigold	98	66.00	16.00	White rust
	Kolar	Mavahalli	Green valley marigold	28	8.00	-	-
		Malandlahalli	Poornima yellow	57	36.00	10.00	White rust
		Mattikunte	Poornima yellow	62	40.00	10.00	-
	Mulbagal	Arati	Poornima white	45	22.00	-	-
		Soorakunte	Green valley marigold	74	48.00	18.00	-
		Gummakullu	Green valley marigold	58	40.00	24.00	-
Average PDI					42.66	19.00	
Chikkaballapur	Gudibande	Bogenahalli	Green valley marigold	50	26.00	-	-
	Chikkaballapur	Hosahudya	Green valley marigold	65	58.00	32.00	-
		Giddaganahalli	Green valley marigold	48	18.00	-	-
		Kurappalli	Green valley marigold	55	28.00	-	-
	Chintamani	Vaddahalli	Green valley marigold	60	32.00	-	-
		Barlahalli	Poornima white	105	66.00	20.00	White rust
Average PDI					40.33	26.00	
Bengaluru rural	Devanahalli	Lalagondahalli	Poornima white	72	40.00	-	-
		Hosakurubarakunte	Poornima white	68	48.00	-	White rust
		Koramangala	Green valley marigold	50	26.00	-	-
Average PDI					38.00	0.00	
Tumkur	Sira	Bargur	Green valley marigold	60	28.00	-	-
	Gubbi	Bukkapatna	Green valley marigold	60	22.00	-	-
		Chikkanahalli	Green valley marigold	125	86.00	14.00	White rust
		Chelur	Poornima white	102	64.00	22.00	White rust
		Hosakere	Green valley marigold	58	30.00	-	-
		Hagalvadi	Green valley marigold	60	44.00	-	-
Average PDI					45.66	18.00	

*DAP- Days after planting

and morphological characters of conidia, the fungus was identified as *Alternaria alternata*. Conidia varied from 22.75 to 63.70 µm in length and 13.65-18.20 µm in width. Conidia had 2-3 transverse septa and usually several longitudinal septa (Ellis, 1971). Basim *et al*. (2017) stated that colonies of *Alternaria* isolates were white-grey mycelium with the mixture of green and dark brown conidia in chains ranged from 7 to 45.9 µm in length.

Table 2. Morphology of *Alternaria alternata* causing leaf blight of chrysanthemum

Conidia		Beak length(µm)	Conidia	Color	No. of septations	
Length (µm)	Width(µm)				Cross	Transverse
33.93-57.42	10.44-18.27	13.05- 26.10	Muriform	Dark brown	6-7	1-3

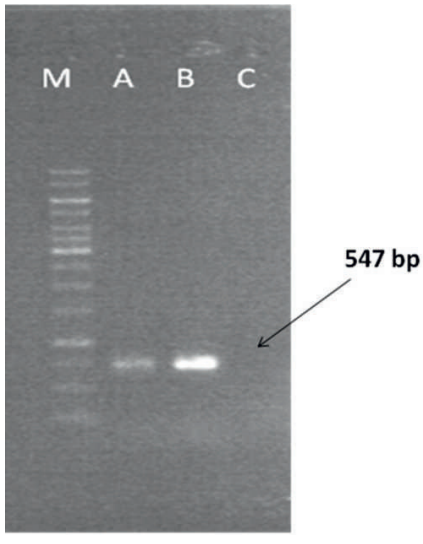


Plate 2. The Agarose gel showing the PCR product of *Alternaria alternata* (Fr.) Keissler M – 1000bp marker; A & B - 547bp amplicons, C- Control

Molecular confirmation of the pathogen

The total genomic DNA was isolated and purified. The genomic DNA was amplifiifed using ITS1 and ITS4 primers, which yielded an amplicon size of 547 bp (Plate 3). After amplification and subsequent confirmation, the PCR product was sequenced. The alignment of the sequence was done using Clustal W and dendrogram was constructed using MEGA X (https;//www.megasoftware.net). The sequence analysis and BLAST search results revealed that the sequence showed 99.30 per cent homology with *Alternaria alternate* infecting *Citrus reticulata* (MH879769). The phylogenetic analysis revealed that *Alternaria alternata* under study had close relationship with Gen Bank accessions MH879769 (Pakistan isolate *Alternaria alternata*) and others *viz.,* MK409081 (USA), JQ625589 (India), MF475920 (Portugal) and DQ323694 (Puerto Rico). Similarly, Shamala and Janardhana (2015) confirmed *A. alternata* causing leaf blight of chrysanthemum using the ITS primers, where they could get an amplicon size of 550bp and subsequent BLAST analysis revealed 99% homology with other isolates.

Effect of fungicides against leaf blight of Chrysanthemum

In vitro* evaluation of fungicides against *A. alternata

Four contact fungicides and three combi products were evaluated at four concentrations i.e. 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm for their efficacy to suppress mycelial growth of *A. alternata* on potato dextrose agar amended with the prescribed concentrations of fungicides by poisoned food technique. All the three combi product fungicides tested have significantly inhibited the mycelial growth of *Alternaria alternata*. The T_5- carbendazim 12% + mancozeb 63% WP, T_6-Zineb 68% + hexaconazole 4%WP, and T_7-Tricyclazole 18% + mancozeb 62%WP were effective at all the concentrations tested (500, 1000, 1500 and 2000 ppm) when compared with contact fungicides but at 1500 ppm and above was highly effective resulting in 100 per cent inhibition of mycelial growth in all the three products tested. Among these three T_6-Zineb 68% + hexaconazole 4%WP, and T_7-Tricyclazole 18% + mancozeb 62%WP were able to give 100 per cent inhibition even at 500 ppm (Table 3).

Among the contact fungicides tested, T_4- Copper oxy chloride 50%WP was highly effective at all the concentrations, with least mycelial growth (6.67 mm) and 92.16 per cent inhibition followed by T_3-Propineb 70% WP with 30.33 mm growth and 63.31 per cent inhibition at 2000 ppm (Table 3). Least inhibition was found with T_2-Chlorothalonil 75%WP (43.14%) with 48.33 mm growth. Similar trend was observed at all the other concentrations tested. Similarly, Shamala and Janardhana

Table 3. *In vitro* evaluation of contact fungicides and combi-products against *Alternaria alternata*

Treatment details	Mycelial growth (mm)							
	Concentration							
	500 ppm	Per cent inhibition	1000 ppm	Per cent inhibition	1500 ppm	Per cent inhibition	2000 ppm	Per cent Inhibition
T_1 Mancozeb 75%WP	76.00 (8.75)	10.59	57.00 (7.58)	32.94	41.33 (6.47)	51.37	37.67 (6.18)	55.69
T_2 Chlorothalonil 75%WP	65.00 (8.09)	23.53	51.67 (7.22)	39.22	50.33 (7.13)	40.78	48.33 (6.99)	43.14
T_3 Propineb70%WP	54.00 (7.38)	36.47	36.33 (6.07)	57.25	31.00 (5.61)	63.53	30.33 (5.55)	64.31
T_4 Copper oxychloride 50%WP	21.33 (4.67)	74.90	13.00 (3.67)	84.71	9.33 (3.14)	89.02	6.67 (2.68)	92.16
T_5 Carbendazim 12% + Mancozeb 63%WP	61.33 (7.86)	27.84	46.33 (6.84)	45.49	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_6 Zineb 68% + Hexaconazole 4% WP	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_7 Tricyclazole 18%+ Mancozeb 62%WP	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_8 Control	85.00 (9.25)	0.00	85.00 (9.25)	0.00	85.00 (9.25)	0.00	85.00 (9.25)	0.00
S.Em±	**1.14**		**2.59**		**0.72**		**0.67**	
CD@ 1%	**4.82**		**10.93**		**3.06**		**2.86**	

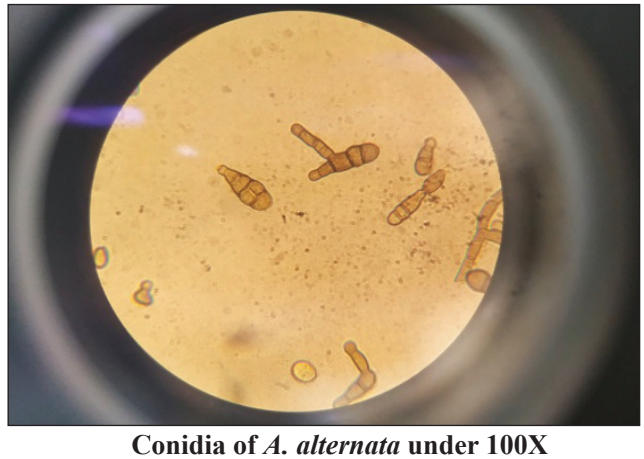
***Values in parenthesis are square root transformed.**

(2015) reported the efficacy of Carbendazim+Mancozeb (2.0%) with 95.65 per cent inhibition followed by Carbendazim at 0.25 per cent concentration with 32.17 per cent inhibition against *Alternaria alternata* of chrysanthemum.

In vitro evaluation of systemic fungicides against *A. alternata*

Three systemic fungicides along with a control were evaluated at four concentrations i.e. 250 ppm, 500 ppm, 750 ppm and 1000 ppm for their efficacy towards inhibition of the mycelial growth of *A. alternata* on potato dextrose agar amended with their prescribed concentrations of fungicides by the poisoned food technique and replicated five times. All the systemic fungicides tested significantly inhibited the mycelia growth of *Alternaria alternata* (Table 5). T_1-Hexaconazole 5% SC and T_3-Tricyclazole 75% WP were very effective at all the concentrations tested and statistically superior over T_2-Azoxystrobin 23% SC (34.40 mm) at 1000 ppm. Devaraja (2011) reported the efficacy of systemic fungicies *viz*., hexaconazole, propiconazole, difenoconazole and penconazole at 0.1 per cent concentrations completely inhibited the mycelial growth of *A. alternata.*

Culture of *A. alternata*



Conidia of *A. alternata* under 100X

Plate 3. Photographs showing culture and conidia of *A. alternata*(Fr.) Keissler

Table 4. *In vitro* evaluation of systemic fungicides against *Alternaria alternata*

Treatment No.	Treatment details	Mycelial growth (mm) Concentration							
		250 ppm	Per cent inhibition	500 ppm	Per cent inhibition	750 ppm	Per cent inhibition	1000 ppm	Per cent inhibition
T_1	Hexaconazole 5%SC	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_2	Azoxystrobin 23% SC	54.20 (7.40)	35.63	48.00 (6.96)	42.99	45.20 (6.76)	46.32	34.40 (5.89)	59.14
T_3	Tricyclazole75%WP	67.80 (8.24)	19.48	23.80 (4.93)	71.73	0.00 (0.71)	100.00	0.00 (0.71)	100.00
T_4	Control	84.20 (9.20)	0.00	84.20 (9.20)	0.00	84.20 (9.20)	0.00	84.20 (9.20)	0.00
	S.Em±	**2.68**		**0.90**		**0.69**		**1.27**	
	CD@ 1%	**11.61**		**3.92**		**3.01**		**5.49**	

***Values in parenthesis are square root transformed.**

In vivo* evaluation of fungicides and a bioagent against *A. alternata

The experiment was conducted to evaluate the relative efficacy of different fungicides and a bioagent as foliar spray against Alternaria leaf blight of chrysanthemum. The experiment was conducted during *kharif/rabi* season of 2018-19 with ten fungicides and one bioagent. Totally three sprays were given at 15 days interval starting from the onset of the disease (40 days after planting). The observations on Alternaria leaf blight was recorded before each spray. Using 0-5 disease scale and converted into per cent disease index (PDI) using the formula given by Wheeler (1969).

From the data it is evident that the T_3- Copper oxy chloride@ 0.3 per cent was very effective in controlling the disease (41.79% disease suppression) with 43.33 per cent disease index and effective in increasing the yield (12.71 q/h) followed by T_5-Zineb 68% + hexaconazole 4%WP (35.82 % disease suppression) with 47.78 per cent disease index and yield of 12.49 q/ha (Table 6). Least disease control of 7.46 per cent was noticed in T_{11}- *Pseudomonas fluorescens* with 68.89 per cent disease index and less yield (5.97 q/ha) and was followed by T_2 – Chlorothalonil @ 0.2 per cent with the PDI of 65.56 and disease suppression of 11.94 per cent with an yield of 6.56 q/ha. The efficacy of Copper oxy chloride @ 0.3 per cent and Chlorothalonil @ 0.2 per cent was well documented by earlier workers, Kamanna *et al*., (2010) and Gangawane (2011). The new product, hexaconazole+zineb @ 0.1% can be used as an alternative to manage the resistance development in the intensive crop production system.

CONCLUSION

The causal organism of leaf blight of Chrysanthemum was found to be *Alternaria alternata* and the highest disease severity was at Chikkanahalli in Tumakuru district and the least disease severity (8%) was recorded at Mavahalli in Kolar district in eastern dry zone of Karnataka. The pathogen produced conidia which are typically muriform, dark brown, thick walled. Majority of conidia are non-beaked few with short rudimentary dark brown beaks. The conidiaum varied in length from 33.93 to 57.42 µm and 10.44 to 18.23 µm. Molecular confirmation of the causal organism was done through PCR amplification and sequencing of ITS region. *In-vitro* evaluation fungicides revealed that, among contact, systemic and combi-products tested Copper oxy chloride 50%WP, Hexaconazole 5% SC and Zineb 68% + hexaconazole 4%WP and Tricyclazole 18% + mancozeb 62%WP respectively were effectively inhibited the pathogen. *In-vivo* evaluation of fungicides revealed, foliar application of Copper oxy chloride 50%WP @ 0.3 per cent and Zineb 68% + hexaconazole 4%WP proved to be highly effective in arresting spread of the disease.

Table 5. *In-vivo* efficacy of different fungicides and a bioagent against *Alternaria* leaf blight of chrysanthemum during *rabi* 2018-19

Treatment No.	Treatment details	Per cent disease index				Per cent disease control After 3rd spray	Yield (q/ha)
		Before spray	After 1st spray	After 2nd spray	After 3rd spray		
T_1	Hexaconazole (0.1%)	43.33 (41.07)*	37.78 (37.71)	50.00 (33.51)	53.33 (46.94)	28.35	7.87
T_2	Chlorothalonil (0.2%)	44.44 (41.75)	44.44 (41.80)	55.56 (36.90)	65.56 (54.14)	11.94	6.56
T_3	Copper oxy chloride (0.3%)	41.11 (39.56)	34.44 (34.35)	45.56 (32.29)	43.33 (41.10)	41.79	12.71
T_4	Mancozeb (0.25%)	25.56 (30.24)	38.89 (38.46)	44.44 (33.21)	54.44 (47.61)	26.86	7.29
T_5	Zineb + Hexaconazole (0.2%)	18.89 (25.62)	34.44 (35.86)	43.33 (35.47)	47.78 (43.70)	35.82	12.49
T_6	Azoxystrobin (0.1%)	36.67 (36.91)	35.56 (36.51)	54.44 (43.49)	52.22 (46.28)	29.85	8.02
T_7	Tricyclazole + Mancozeb (0.2%)	33.33 (35.00)	48.89 (44.36)	45.56 (37.07)	52.22 (46.28)	29.85	9.52
T_8	Carbendazim + Mancozeb (0.2%)	38.89 (38.34)	37.78 (37.14)	51.11 (37.65)	53.33 (47.08)	28.35	7.83
T_9	Tricyclazole (0.1%)	43.33 (41.14)	48.89 (44.36)	54.44 (41.71)	62.22 (52.09)	16.41	6.88
T_{10}	Propineb (0.2%)	44.44 (41.75)	52.22 (46.33)	54.44 (43.68)	61.11 (51.65)	17.91	6.96
T_{11}	*Pseudomonas fluorescens* (1.0%)	28.89 (32.41)	51.11 (45.64)	55.56 (45.07)	68.89 (56.47)	7.46	5.97
T_{12}	Control	41.11 (39.87)	50.00 (44.95)	61.11 (48.25)	74.44 (59.76)	0.00	5.20
	S.Em±	**5.202**	**4.947**	**3.987**	**6.65**		**2.99**
	CD@ 0.05%	**15.25**	**14.51**	**15.89**	**19.51**		**8.78**

*Values in parenthesis are arcsine transformed

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