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Evaluation and bio-efficacy of fungicides, botanicals and bioagents against onion twister blight disease caused by *Colletotrichum gloeosporioides* in Andhra Pradesh

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Abstract
Onion (*Allium cepa* L.) is a widely cultivated bulbous vegetable crop appreciated globally for its strong taste and distinct flavour. The crop was infected by plethora of economically important diseases. One among these diseases is twister blight, caused by *Colletotrichum gloeosporioides* resulting in substantial yield losses. Due to the scarcity of available information on disease management aspects, a study was emphasized for the management of disease under *in vitro* conditions using different fungicides, botanicals and bioagents at different concentrations. Among the seven fungicides evaluated, propiconazole, tebuconazole + trifloxystrobin and kresoxim-methyl at tested concentrations were proved to be effective in inhibiting the pathogen. Among the different botanicals tested, lemongrass oil at 15 per cent concentration was found to be most effective, showing cent per cent mycelial growth inhibition. *Bacillus subtilis* and *Trichoderma reesei* were noted as most effective among the tested bioagents with 87.11 and 75.31 per cent inhibition rate respectively.

Keywords: *Colletotrichum gloeosporioides*, fungicides, botanicals, bioagents

Introduction
Onion (*Allium cepa* L.), commonly referred to as the "queen of kitchen," is one of India's oldest recognized and most important vegetable crops. India stands as the world's second-largest onion producer, boosting a remarkable production of 22.07 million metric tons and an impressive productivity of 16.78 tons per hectare. (Kumawat and Raheman, 2022) [5]. Despite its significance, onions are prone to many diseases, including purple blotch, twister blight, botrytis leaf blight, bulb rot, downy mildew, pink rot, soft rot and white rot (Oduro, 2000; Offei *et al.*, 2008) [7, 8]. Twister blight disease of onion ranks as one of the most destructive foliar diseases affecting the crop. The discovery of this disease dates back to 1969 in the vicinity of Zaria, Nigeria. The disease is incited by the fungal pathogen *Colletotrichum gloeosporioides* (Ebenebe, 1980) [3]. Due to the scarcity of information concerning the disease outbreak, it was imperative to conduct a study in order to develop effective disease management strategies. As a result, the present experiment was conducted on the management of the disease under i*n vitro* conditions.

Materials and Methods
Poison food technique of fungicides: The study was carried out at department of Plant Pathology, Dr. YSRHU-COH, Anantharajupeta, during the year 2022-23. Seven fungicides *viz.*, chlorothalonil, metiram + pyraclostrobin, azoxystrobin, propiconazole, fluxapyroxad + pyraclostrobin, tebuconazole + trifloxystrobin and kresoxim-methyl each at three different concentrations were evaluated by poisoned food technique (Nene and Thapliyal, 1982) [6]. In order to obtain the necessary concentration of the fungicide, the required amount of each fungicide was mixed separately into molten and cold potato dextrose agar. Subsequently, 20 ml of the poisoned medium was transferred into sterile Petri plates. A 5 mm mycelial disc from an actively growing fungus culture was cut using a sterile cork borer and placed at the middle of each poisoned agar plate. Without adding any fungicide to the medium, control was maintained. Each experimental treatment was replicated thrice.

These plates were then incubated at 25±2 ℃ and then the radial diameter of the colony was measured. By comparing the inhibition of mycelial growth to control, a fungicide's effectiveness was estimated using a formula given by Vincent (1947) [15].

$$\text{Percent inhibition (PI)} = \frac{C-T}{C} \times 100$$

Where, PI= Per cent inhibition
C= Colony diameter in control (mm)
T= Colony diameter in treatment (mm)

Poison food technique of botanicals: Fresh plant parts were collected from medicinal block of Dr. YSRHU-COH, Anantharajupeta. Plant extracts were prepared by following the method described by Jadeja, 2003 [4]. A hundred grams fresh sample of ten different botanicals *viz*., *Azadirachta indica*, *Catharanthus roseus*, *Ocimum sanctum*, *Bixa orellana*, *Andrographis paniculata*, *Cymbopogon citratus*, *Lantana camara*, *Solanum nigrum*, *Withania somnifera* and *Eucalyptus globulus* were collected and crushed by adding 100 ml of sterile distilled water (1:1 w/v). The extract was then filtered using Whatman filter paper. Finally, the filtrate obtained was utilized as a stock solution. The prepared solution was diluted to required concentrations. The extracts were examined on an appropriate medium at three distinct concentrations *viz*., 5%, 10% and 15% to assess their efficacy against the pathogen using poisoned food technique under *in vitro* conditions.

Twenty ml of the poisoned medium was poured into each of the 90 mm sterilized petriplates. Each plate was inoculated with mycelium of five mm discs cut from the periphery of an actively growing culture using a cork borer. One such disc was carefully positioned at the center of each agar plate. The pathogen was grown on PDA plates to maintain as a control. These plates were subsequently incubated at a temperature of 25±2 ℃ for a period of ten days. Radial growth measurements were recorded when the pathogen exhibited its maximum growth on the control plates. The efficacy of botanicals was represented as a percentage of radial growth compared to the control which was calculated by using the formula proposed by Vincent (1947) [15].

$$\text{Percent inhibition (PI)} = \frac{C-T}{C} \times 100$$

Where, PI= Per cent inhibition
C= Colony diameter in control (mm)
T= Colony diameter in treatment (mm)

Dual culture technique of bioagents: Six biocontrol agents *i.e.*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma reesei* and *Trichoderma longibrachiatum* were collected from department of Plant Pathology at Dr. YSRHU-COH, Anantharajupeta. Bioagents were employed against the test pathogen to study its efficacy using dual culture technique.

Twenty ml of sterilised and cooled potato dextrose agar was poured into sterile petri dishes and left to solidify. To assess the effectiveness of the fungal biocontrol agent, mycelial discs of the test fungus were placed at one end of the petri dish, while the antagonistic fungus was positioned at the opposite end. When assessing the bacterial antagonist, the bacterium was streaked along the edges of the petri dishes and mycelial discs of the fungus was positioned at the centre. Each treatment was maintained with three replications. The plates were incubated at 25±2 ℃ and inhibition zones were measured by measuring the distance between the margins of the test fungus and the antagonistic bioagent. Per cent growth inhibition of the pathogen was determined using the formula suggested by Vincent (1947) [15].

$$\text{Percent inhibition (PI)} = \frac{C-T}{C} \times 100$$

Where, PI= Per cent inhibition
C= Colony diameter in control (mm)
T= Colony diameter in treatment (mm)

Results and Discussion

Fungicides: *In vitro* evaluation of fungicides provide useful preliminary information regarding its efficacy against a pathogen. Three fungicides, namely propiconazole, tebuconazole + trifloxystrobin and kresoxim methyl were shown to be the most effective when compared to the other tested fungicides. These three fungicides reported a 100% suppression of mean mycelial growth at all specified concentrations. It was subsequently followed by a 74.09% inhibition rate with azoxystrobin at a concentration of 0.5%, noticeably on par with chlorothalonil mycelial inhibition rates of 73.25%, 72.67% and 72.38% at concentrations of 0.2%, 0.15%, and 0.1% respectively (Table 1 and Plate 1).

Similar experiments were performed by various researchers *viz*., Parvathy and Girija (2016) [9], Vandana *et al.* (2021) [14] on chemical control of the pathogen and stated that Propiconazole and kresoxim-methyl exhibited cent per cent mycelial inhibition respectively. propiconazole 25% EC, a triazole group of fungicide, functions by inhibiting the activity of 14-α-sterol demethylase leading to prevention of biosynthesis of ergosterols which are the essential component of fungal cell membrane, thereby death of the fungus occurs. Kresoxim methyl 44.3% EC a member of the strobilurin fungicide class, acts by inhibiting fungal respiration through its binding to the cytochrome complex II. Additionally, it disrupts fungal metabolism. Results were in accordance with the findings of Shedge *et al.* (2021) [13] who proved that azoxystrobin was least effective in inhibiting the mycelial growth.

Table 1: *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides* causing twister blight of onion

S. No.	Fungicides	Concentration (per cent)	*Colletotrichum gloeosporioides*	
			Mean diameter of colony (mm)*	Per cent inhibition over control
1	Chlorothalonil 75%WP	0.1	23.75	72.38^{bc} (58.29)
		0.15	23.5	72.67^{bc} (58.48)
		0.20	23.0	73.25^{bc} (58.85)
2	Metiram 55% + Pyraclostrobin 5% WG	0.2	26.95	68.66^{ef} (55.95)
		0.25	25	70.93^{de} (57.37)
		0.3	24.85	71.10^{de} (57.48)
3	Azoxystrobin 23% EC	0.1	26.66	69.00^{e} (56.16)
		0.25	24.51	71.5^{cd} (57.73)
		0.50	22.28	74.09^{b} (59.40)
4	Propiconazole 25% EC	0.1	0	100^{a} (90.00)
		0.15	0	100^{a} (90.00)
		0.2	0	100^{a} (90.00)
5	Fluxapyroxad 25% + Pyraclostrobin 25% SC	0.02	33.75	60.75^{gh} (51.20)
		0.04	32.33	62.4^{g} (52.18)
		0.05	29.45	65.75^{f} (54.18)
6	Tebuconazole 50% + Trifloxystrobin 25% WG	0.02	0	100^{a} (90.00)
		0.04	0	100^{a} (90.00)
		0.05	0	100^{a} (90.00)
7	Kresoxim-methyl 44.3% SC	0.02	0	100 ^{a} (90.00)
		0.06	0	100 ^{a} (90.00)
		0.08	0	100 ^{a} (90.00)
8	Control	-	86	-
		CD at 5%	2.98	
		SE(d)±	1.47	
		SE(m)±	1.04	

*Average of three replications

Figures in the parenthesis are arc sine transformed values

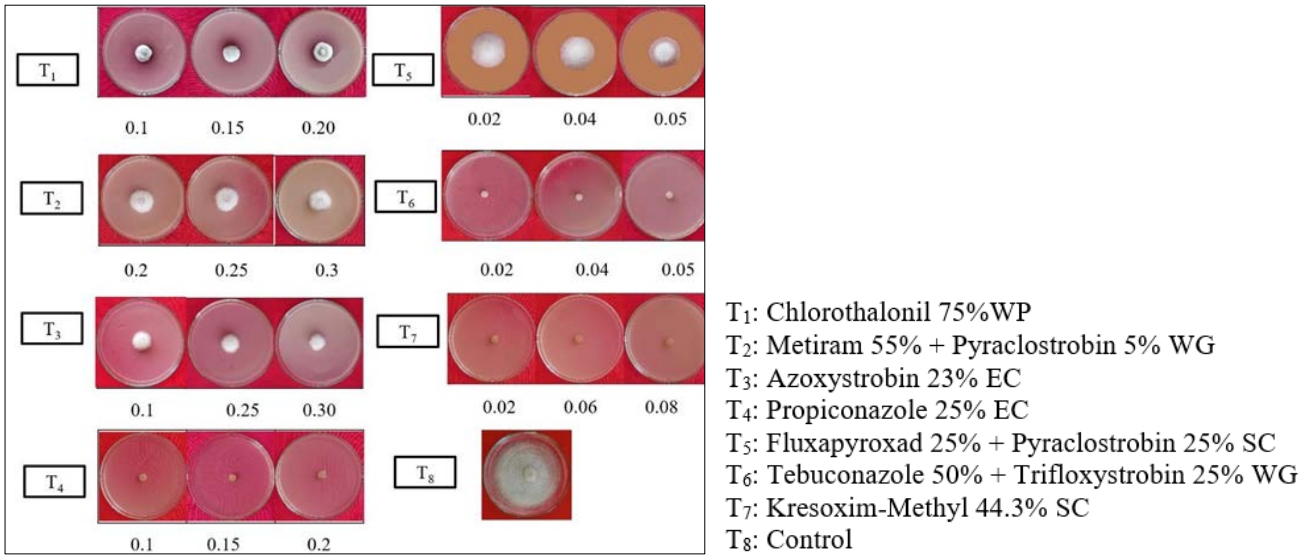

Plate 1: *In vitro* evaluation of different fungicides against *Colletotrichum gloeosporioides* causing twister blight in onion

Botanicals: In this current study, ten plant extracts were assessed against *C. gloeosporioides* under *in vitro* conditions. Results confirmed that all the phytoextracts inhibited the growth of the pathogen significantly. Among different botanicals tested, lemon grass oil at 15% concentration resulted in complete inhibition of the pathogen which was followed by lemon grass oil at 10% concentration (98.23%). Minimum per cent inhibition was recorded with eucalyptus at 5% concentration (39.81%), (Table 2 and Plate 2).

The development of fungal mycelium was significantly inhibited by lemongrass oil, known for its antifungal properties due to the presence of citral content. The fungicidal spectrum of neem, *Azadirachta indica* has been associated with azadiractrachin, a member of the C25 terpenoids group, as strongly supported by Raheja and Thakore (2002) [10]. The findings were similar with the reports of Chala *et al.* (2014) indicating that lemon grass oil exhibited cent per cent inhibition of *C. gloeosporioides.*

Table 2: *In vitro* evaluation of different phyto-extracts against *Colletotrichum gloeosporioides* causing twister blight in onion

S. No.	Phyto-extracts	Per cent growth inhibition*			Mean
		Per cent concentrations			
		05	10	15	
1.	Neem	56.68^{j} (48.83)	60.15^{hi} (50.86)	62.02^{h} (51.95)	59.61^{d} (50.55)
2.	Periwinkle	52.5^{kl} (46.43)	56.23^{j} (48.58)	60.35^{hi} (50.98)	56.36^{e} (48.66)
3.	Tulasi	61.91^{hi} (51.90)	64.5^{fg} (53.42)	66.33^{f} (54.53)	64.25^{c} (53.29)
4.	Bixa	47.5^{lm} (43.57)	48.35^{lm} (44.05)	50.5^{l} (45.29)	48.78^{g} (44.30)
5.	Kalmegh	50.08^{l} (45.04)	52.67^{k} (46.53)	54.94^{jk} (47.83)	52.56^{f} (46.47)
6.	Lemon grass	96.2^{b} (78.75)	98.5^{ab} (82.97)	100^{a} (90.00)	98.23^{a} (83.91)
7.	Lantana	40.62^{pq} (39.60)	42.32^{op} (40.59)	45.8^{mn} (42.60)	42.91^{i} (40.93)
8.	Black night shade	42.46^{o} (40.67)	43.27^{no} (41.13)	46.86^{lmn} (43.20)	44.20^{h} (41.66)
9.	Ashwagandha	77.03^{e} (61.37)	80.52^{cd} (63.80)	81.68^{c} (64.65)	79.74^{b} (63.27)
10.	Eucalyptus	38.06^{qr} (38.10)	39.5^{pqr} (38.93)	41.86^{opq} (40.31)	39.81^{j} (39.11)
	Mean	56.30 (49.43)	58.60 (51.09)	61.03 (53.13)	
	S. Em. ±		0.86		
	C. D. at 5%		2.43		
	C. V. %		2.57		

*Average of three replications

Figures in the parenthesis are arc sine transformed values

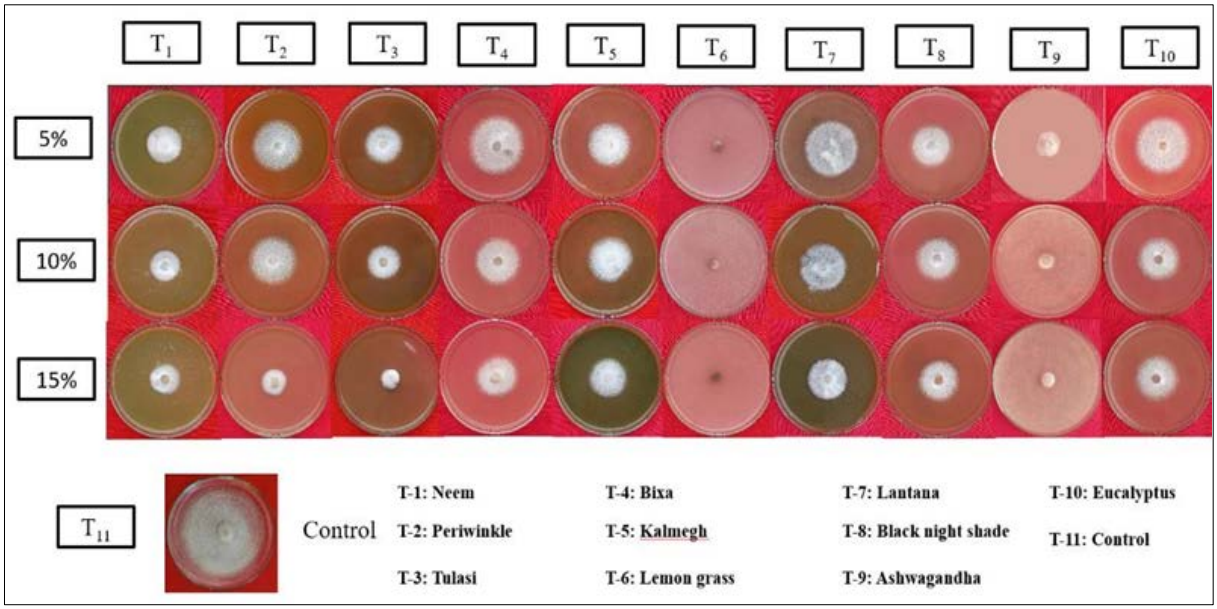

Plate 2: *In vitro* evaluation of different phyto-extracts against *Colletotrichum gloeosporioides* causing twister blight in onion

Bioagents: Four bacterial and fungal biocontrol agents were examined against *C. gloeosporioides* in the present study. Findings revealed that all of the antagonists greatly inhibited the development of the fungus. Maximum colony growth reduction was noticed in *Bacillus subtilis* (87.11 mm) among the bacterial bioagents and *Trichoderma reesei* (75.31 mm) among the fungal bioagents which were significantly superior to all the bioagents tested.), (Table 3 and Plate 3). The fungicidal or fungistatic effects of these bioagents are the consequence of a number of mechanisms, including antibiosis, lysis, mycoparasitism, competition, and the production of both volatile and non-volatile chemicals. The T1 and T2 isolates of *T. harzianum* were found to be effective at preventing the growth of *Colletotrichum gloeosporioides* as reported by Santha Kumari (2002) [12].

According to Robinson and Park (1966) [11] and Dennis and Webster (1971) [2], the synthesis of the acetaldehyde compound is the main cause of *Trichoderma* spp. antagonistic behavior against numerous fungi. This could possibly be the cause of its adverse impact on *C. gloeosporioides.*

Table 3. *In vitro* evaluation of bioagents against *Colletotrichum gloeosporioides* causing twister blight of onion

S. No	Bioagent	Radial growth of mycelium (mm)*	Per cent inhibition over control (%)
1	*Trichoderma harzianum*	57.30	36.33^{g} (37.06)
2	*Trichoderma reesei*	22.22	75.31^{b} (60.20)
3	*Trichoderma longibrachiatum*	50.60	43.78^{e} (41.42)
4	*Trichoderma viride*	36.66	59.26^{c} (50.33)
5	*Pseudomonas fluorescens*	40.00	55.55^{d} (48.18)
6	*Pseudomonas aeruginosa*	52.00	42.22^{ef} (40.52)
7	*Bacillus subtilis*	11.60	87.11^{a} (68.95)

8	*Bacillus cereus*	54.50	39.44^{fg} (38.90)
9	Control	90.00	-
	C.D at 5%		3.366
	SE(m)±		1.113
	SE(d) ±		1.574
	C.V%		3.513

*Average of three replications

Figures in the parenthesis are arc sine transformed values

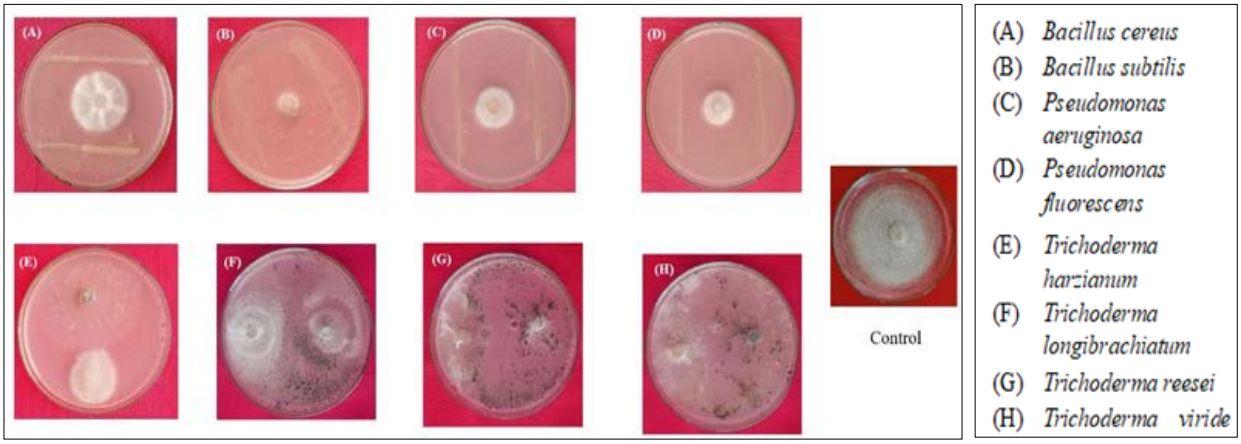

Plate 3: *In vitro* efficacy of bioagents against *Colletotrichum gloeospoiriodes* causing twister blight in onion

Conclusion

In vitro poisoned food technique was performed to examine the effectiveness of seven fungicides and ten botanicals, while dual culture was conducted to determine the efficacy of bioagents, against *C. Gloeosporioides*. Results divulged that propiconazole, tebuconazole + trifloxystrobin and kresoxim-methyl each at all the concentrations were significantly superior (100%) over all other treatments in inhibiting the growth of *C. gloeosporioides*. Among the botanicals, lemongrass oil at 15% concentration was effective in inhibiting the growth of the test fungus (100%). *Bacillus subtilis* (87.11%) and *Trichoderma reesei* (75.31%) performed best in inhibiting the pathogen among the tested bioagents.

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