

EFFICACY OF BIO-INOCULANTS AND NEW GENERATION FUNGICIDES FOR THE MANAGEMENT OF TWISTER BLIGHT OF ONION CAUSED BY *COLLETOTRICHUM GLOEOSPORIOIDES*

K. Yamunarani¹, P. Mareeswari^{2*}, A. Kalyanasundaram³, S. Sangeetha⁴, J. Marimuthu⁵, D. Jayaraj⁶ & R. Naveen Kumar⁷

^{1&3}- Department of Plant protection, ⁴Department of Social Science, ^{1,3 & 4}Horticultural College and Research Institute for Women, TNAU, Tiruchirappalli -620027, Tamil Nadu, India

²-Department of Plant Pathology, Agricultural College and Research Institute, Madurai-625104, Tamil Nadu, India

⁵-Department of Vegetable Science, Horticultural College and Research Institute, TNAU, Coimbatore- 641003, Tamil Nadu, India

^{6&7}-Department of Plantation, Spices, Medicinal and Aromatic crops, Horticultural College and Research Institute, TNAU, Coimbatore- 641003, Tamil Nadu, India

*Corresponding Author: P.Mareeswari, mareeswari.p@tnau.ac.in

ABSTRACT

Onion (*Allium cepa* L.) is an important vegetable crop worldwide and has great economic importance because of its nutritional and medicinal values. Among the diseases, the anthracnose-twister blight plays a vital role in its low productivity which at present is considered to be the most destructive disease of onion in the country. The chemical control based on the use of fungicides is most effective and reliable method. New fungicides with novel chemistry are being introduced and evaluated for plant disease control. The present study was carried out to formulate integrated approach by combining newer chemical with biocontrol agents. Native isolates of Twister blight of onion caused by *Colletotrichum gloeosporioides* was collected from onion growing areas of Pullambadi block of Trichy district. For *in vitro* screening *Bacillus subtilis* (Bbv57) and *Trichoderma asperellum* (TNAU Tv1) was used as per the recommendation along with Pyraclostrobin, Fusilazole, Azoxystrobin and Metiram along with Copper oxychloride as chemical check. Efficacy of different fungicides viz., Pyraclostrobin 0.1%, Fusilazole 0.1%, Azoxystrobin 0.1%, Metiram 0.1 % along with Copper oxychloride 0.25 % was evaluated against *Colletotrichum gloeosporioides* causing twister blight of onion using poisoned food technique. Among the new generation fungicides tested, Fusilazole 0.1% showed cent percent inhibition of mycelial growth and copper oxy chloride recorded highest inhibition of 74.11% mycelial growth and was significantly superior over all other treatments. Pyraclostrobin, Azoxystrobin and Metiram (systemic fungicides) recorded partial inhibition of mycelial growth of 68.6 %, 7.40 % and 55.6% respectively. In dual culture technique the efficacy of *Bacillus subtilis* (Bbv57) and *Trichoderma asperellum* (TNAU Tv1) was studied. Among the two *Trichoderma asperellum* (TNAU Tv1) exhibited 100 % inhibition and parasitization of *Colletotrichum gloeosporioides* whereas *Bacillus subtilis* (Bbv57) exhibited 93.85 % inhibition over control.

KEYWORDS: biological control, new generation fungicide, onion twister blight

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important bulb vegetables cultivated worldwide and has considerable economic, nutritional and medicinal importance. It is an essential component of the human diet and is valued for its characteristic flavour and diverse bioactive compounds. Onion possesses several medicinal properties, including antioxidant, anti-inflammatory, analgesic and hypocholesterolemic activities, which contribute to its importance as a functional food (1). Despite its economic significance, onion production is affected by several biotic and abiotic stresses, among which fungal diseases are major constraints to productivity and bulb quality. Among the important fungal diseases, anthracnose-twister disease, commonly known as twister blight, is a serious constraint to onion production and can cause considerable yield losses under favourable environmental conditions.

Twister blight of onion is caused by *Colletotrichum gloeosporioides* and is characterized by water-soaked lesions that subsequently develop orange-coloured masses arranged in concentric rings, followed by the formation of black acervuli and twisting of the leaves from the neck region. Severe infection may result in leaf dieback and premature drying, thereby reducing the photosynthetic capacity of the plant and ultimately affecting bulb development and yield. In the present study, the pathogenicity test confirmed that the native isolate was highly pathogenic to onion, producing typical disease symptoms within 72 hours after inoculation and recording 100 per cent disease incidence.

Management of twister blight generally involves cultural practices, sanitation, healthy planting material, biological control agents and chemical fungicides. Among these approaches, fungicides remain an important and reliable method for reducing disease development. However, continuous dependence on conventional fungicides may increase the risk of fungicide resistance, environmental contamination and disruption of beneficial microorganisms. Therefore, evaluation of new-generation fungicides with different modes of action is essential for identifying effective alternatives for disease management. Previous studies have also demonstrated the potential of fungicides in reducing the severity of anthracnose-twister disease of onion (6,7).

Biological control represents an important component of sustainable disease management. Beneficial microorganisms such as *Bacillus subtilis* and *Trichoderma asperellum* can suppress fungal pathogens through antagonistic mechanisms, including competition, antibiosis and mycoparasitism. Integration of biological control agents with effective fungicides may provide more reliable disease suppression while reducing excessive dependence on chemical inputs. In the present investigation, *B. subtilis* (Bbv57) and *T. asperellum* (TNAU Tv1) were evaluated against *C. gloeosporioides* along with the new-generation fungicides pyraclostrobin, fusilazole, azoxystrobin and metiram, with copper oxychloride included as the chemical check.

The need for effective management strategies is particularly important because the pathogen can cause rapid disease development under favourable conditions. Hence, identification of highly effective bioinoculants and fungicides through *in vitro*, pot culture and field evaluation can provide a sound basis for developing practical management recommendations. Therefore, the present study was undertaken to evaluate the efficacy of selected bioinoculants and new-generation fungicides against twister blight of onion caused by *Colletotrichum gloeosporioides* and to identify effective treatments for sustainable disease management and improved bulb yield.

MATERIALS AND METHODS

In vitro screening of bioagents and fungicides

Native isolates of Twister blight of onion caused by *Colletotrichum gloeosporioides* was collected from onion growing areas of Pullambadi block of Trichy district. The collected isolates was screened, characterized and identified as *Colletotrichum gloeosporioides*. For *in vitro* screening *Bacillus subtilis* (Bbv57) and *Trichoderma asperellum* (TNAU Tv1) was used as per the recommendation along with Pyraclostrobin, Fusilazole, Azoxystrobin and Metiram along with Copper oxychloride as chemical check. Three replications were maintained for each treatment. The results were expressed as per cent inhibition over control by using the formula.

Pathogenicity test

Onion plants infected with twister blight disease were collected from onion growing areas in Pullambadi block of Trichy district. The target pathogen *Colletotrichum gloeosporioides* was isolated from the infected onion plant showing obvious symptoms like the presence of sunken lesion with orange to black masses of mycelia on the leaves and twisting from the neck. It was then subjected to pure culture production and afterwards, mass produced. Subsequently, it was once tested for its pathogenicity to the onion cultivar where it was isolated. Ten test plant samples per pathogen were used. Before inoculation, the spore densities of the pathogens was standardized at 2.5×10^6 before 25 μ l of the fungal suspensions were dropped in four inoculation points i.e. two in the leaf and two in the neck. The host plants were observed for symptom development. Koch's Postulates were satisfied after the pathogens were re-isolated from the host plants and re-checked for morphological characteristics.

Pot Experiment

Onion seedlings (CO 5) were grown in pots (three plant/ pot). Fourteen-day old sporulating cultures of the pathogens were flooded with 100 mL sterile distilled water and the surface growth was scraped. The spore suspension was filtered through four layers of cheesecloth to separate the mycelia from the spores. The spore densities (2.5×10^6) was adjusted using a haemocytometer. The potted onion plants were divided into eight treatments and three replications as follows:

Table 1. Treatment details under pot culture.

T ₁	<i>Bacillus subtilis</i> (Bbv57) 2.5 kg / ha basal application + 10 g/kg bulb treatment + 0.5 % Foliar spray (30 & 45 days after planting)
T ₂	<i>Trichoderma asperellum</i> (TNAU Tv1) 10g / kg 2.5kg/ha basal application + 4 g/kg bulb treatment + 2 % Foliar spray (30 & 45 days after planting)
T ₃	Pyraclostrobin 0.1% bulb treatment + 0.1 % Foliar spray (30 & 45 days after planting)
T ₄	Fusilazole 0.1% bulb treatment + 0.1 % Foliar spray (30 & 45 days after planting)
T ₅	Azoxystrobin 0.1% bulb treatment + 0.1 % Foliar spray (30 & 45 days after planting)
T ₆	Metiram 0.1 %. bulb treatment + 0.1 % Foliar spray (30 & 45 days after planting)
T ₇	Copper oxychloride 0.25 % - bulb treatment + Foliar spray (recommended chemical check) (30 & 45 days after planting)
T ₈	Untreated control

Table 2. List of fungicides used for *in vitro* evaluation

Sl. No.	Common name	Trade name
1	Pyraclostrobin 20 % WG	Velocity
2	Fusilazole 40 % EC	Cursor
3	Azoxystrobin 23 % SC	Amistar
4	Metiram 70 % WG	Polyram
5	Copper oxychloride 88% WP	Blue Copper

The plants were spray inoculated with fungal suspension and immediately covered with a plastic bag for overnight incubation to maintain high relative humidity. The plastic covers were removed early in the morning to prevent the plant

from heat stress and put in place again late in the afternoon. High relative humidity was maintained during the day for three consecutive days. Plants were covered with a plastic bag for three straight nights for symptom development. Six (6) days after inoculation, the most promising fungicides in the previously conducted *in-vitro* experiments were used viz., Pyraclostrobin 0.1%, Fusilazole 0.1%, Azoxystrobin 0.1%, Metiram 0.1 % along with Copper oxychloride 0.25 % was evaluated against *Colletotrichum gloeosporioides* in the infected potted onion plants (Tables 1 and 2). The chemical treatments were sprayed on the surface of the previously inoculated leaves using hand-held sprayer. Sterile distilled water was used in the untreated control plants. Plants were placed in an elevated platform inside the screen house at temperature ranging from 28°C-29°C. The set up was arranged in CRD with three replications. Plants were maintained based on onion's recommended cultural management practices, and the insect pests were managed through hand picking. The data were analyzed by ANOVA using SAS v.9.1 software. Critical differences among treatments were calculated at 5% probability level of significance using Tukey's Studentized Range (HSD) Test. Disease evaluation was carried out a day before the next spraying schedule and treatments were applied two times at 15 days interval for a total of 3 applications. Disease development on the leaves was scored on 0-9 scale at 15 days after inoculation Tables 3.

Table 3. Rating scale for *Colletotrichum gloeosporioides* infected plants, disease development on the leaves scored (Alberto 2014)

Scale	Description
0	no symptoms
1	1-5 small, whitish water soaked lesions
3	1-2 big, whitish water soaked lesions
5	1-2 prominent lesions with orange fungal growth at the middle of the lesions
7	1 significant lesion with orange acervuli arranged in concentric rings at the centre of the lesions, leaf turn yellowish green
9	1 significant lesion with black acervuli arranged in concentric rings at the middle of the lesions, leaf turn yellow

Gathered Data

A week after inoculation, the following parameters were taken and observed for the length of neck and disease development.

$$\text{Disease Incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants}} \times 100$$

$$\text{Disease Severity (\%)} = \frac{n(1)+n(3)+n(5)+n(7)+n(9)}{N \times 9} \times 100$$

Where:

n = no. of infected leaves classified by grade

N = total number of leaves/pot examined

Length of the neck— this was taken by measuring the length of the neck, from the base of the leaves of each individual onion plants using a ruler (cm).

Yield (g)—this was taken using a weighing scale.

RESULT AND DISCUSSION

Pathogenicity test disease incidence(%)

Colletotrichum gloeosporioides inoculated plants showed symptoms in onion at 72 hours after inoculation. The symptoms of onion plants inoculated with *Colletotrichum gloeosporioides* started as water soaked lesion until it further developed into an orange masses arranged in concentric rings and later turned into black acervulus as it matures and showed slight twisting from the neck. Other affected leaves showed dieback and eventually die (Fig. 1). The inoculated isolates were found to be highly pathogenic to onion plants (100% disease incidence) as it shows the typical symptoms occurring in the field.



Fig.1–(a) Twisting symptoms in onion plants inoculated with (*Colletotrichum gloeosporioides*).

Efficacy of different fungicides

Efficacy of different fungicides viz., Pyraclostrobin 0.1%, Fusilazole 0.1%, Azoxystrobin 0.1%, Metiram 0.1 % along with Copper oxychloride 0.25 % was evaluated against *Colletotrichum gloeosporioides* causing twister blight of onion using poisoned food technique. Among the new generation fungicides tested, Fusilazole 0.1% showed cent percent inhibition of mycelial growth and copper oxy chloride recorded the highest inhibition of 74.11% mycelial growth and was significantly superior over all other treatments. Pyraclostrobin, Azoxystrobin and Metiram (systemic fungicides) recorded partial inhibition of mycelial growth of 68.6 %, 7.40 % and 55.6% respectively. In dual culture technique the efficacy of *Bacillus subtilis* (Bbv57) and *Trichoderma asperellum* (TNAU Tv1) was studied. Among the two *Trichoderma asperellum* (TNAU Tv1) exhibited 100 % inhibition and parasitization of *Colletotrichum gloeosporioides* was observed whereas *Bacillus subtilis* (TNAU) exhibited 93.85 % inhibition over control (Table 4).

Table 4. *In vitro* evaluation of new generation fungicides against *Colletotrichum gloeosporioides*

S.No.	Treatment details	Mycelial growth (mm)	Percent Inhibition over control
T ₁	<i>Bacillus subtilis</i> (Bbv57)	5.5	93.8 (75.58)*
T ₂	<i>Trichoderma asperellum</i> (TNAU Tv1)	0.0	100 (90.00)
T ₃	Pyraclostrobin 0.1%	28.3	68.60 (55.90)
T ₄	Fusilazole 0.1%	0.0	100 (90.00)
T ₅	Azoxystrobin 0.1%	83.3	7.40 (15.18)
T ₆	Metiram 0.1 %.	55.6	55.60 (48.20)
T ₇	Copper oxychloride 0.25 %.	23.3	74.11 (59.40)
T ₈	Untreated control	90.00	
		S. Em. ±	0.62
		CD(0.01)	1.91

Figure in parentheses are Arcsine transformed values

Potculture experiment was conducted at HC&RI (W), Trichy with Onion variety CO 5. The experiment was conducted in completely randomized design with 8 treatments and each treatment replicated three times. The best bio-inoculants was applied as seed treatment and soil application along with recommended chemical check and untreated control. Among the treatments tested, Fusilazole 0.1 percent showed 83.92 percent inhibition of disease and copper oxy chloride recorded 80 percent inhibition of disease and was significantly different. *Trichoderma asperellum* (TNAU) exhibited 80 percent inhibition of disease over control. Similar results were obtained by (6) lowest percentage of disease severity was observed in onion plants spray inoculated with *Colletotrichum gloeosporioides* and treated with fungicides. The response of onion plants when treated with fungicide as protective spray had the least disease severity.

In field trial bulb treatment and foliar spray of Fusilazole 0.1 percent showed 44.12 percent inhibition of disease with highest yield of 13.9 t/ha and 1.89 BCR. Similarly, *Trichoderma asperellum* exhibited 38 percent inhibition of disease over control with yield of 12.7 t/ha and 1.72 BCR. Copper oxy chloride recorded 36.47 percent inhibition of disease with yield of 12 t/ha and 1.63 BCR (Table 5). Triazoles are new class of fungicides, belong to Group 3 fungicide family, involved in ergosterol biosynthesis inhibition (EBI) that prevents the formation of a key fungal cell membrane component (7). Thus, its use improved the plant disease resistance and played an essential role in maximizing benefits. Flusilazole is a systemic fungicide that demonstrates potential in controlling onion twister blight. It works by inhibiting the biosynthesis of ergosterol, a vital component of fungal cell membranes, leading to the death of the fungus. Flusilazole has a broad spectrum of activity against various fungi, including those that cause twister blight and also it is considered safe for beneficial insects, such as bees and ladybugs, important for maintaining a healthy ecosystem. Similar results were obtained by (6; 7; 8)

Table 5. Efficacy of new generation fungicides against *Colletotrichum gloeosporioides* under pot culture experiment

S.No	Treatment details	PDI at 15 days after first spray	Percent Reduction over control	PDI at 15 days after second spray	Percent Reduction over control	PDI at 15 days before harvesting	Percent Reduction over control
T ₁	<i>Bacillus subtilis</i> (Bbv57)	4.00 (11.53)	78.04	8.00 (16.42)	68.95	10.7 (19.09)	77.06
T ₂	<i>Trichoderma asperellum</i> (TNAU Tv1)	3.55 (10.86)	80.51	5.33 (13.34)	79.31	9.33 (17.78)	80.00
T ₃	Pyraclostrobin 0.1%	4.44 (12.16)	75.63	14.66 (22.51)	43.11	24.88 (29.92)	46.67
T ₄	Fusilazole 0.1%	1.77 (7.64)	90.28	3.50 (10.78)	86.41	7.50 (15.89)	83.92
T ₅	Azoxystrobin 0.1%	13.33 (21.41)	26.83	22.66 (28.42)	12.06	35.5 (36.57)	23.91
T ₆	Metiram 0.1 %.	4.00 (11.53)	78.04	6.66 (14.95)	74.15	12.22 (20.46)	73.81
T ₇	Copper oxychloride 0.25 %.	2.22 (8.56)	87.81	4.00 (11.53)	84.47	9.33 (17.78)	80.00
T ₈	Untreated control	18.22 (25.26)		25.77 (30.50)		46.66 (43.08)	
CD(0.01)		0.47		1.23		1.24	
		4.23		6.53		4.95	

CONCLUSION

The present study demonstrated the potential of biological control agents and new-generation fungicides for managing onion twister blight caused by *Colletotrichum gloeosporioides*. The pathogen produced characteristic symptoms with 100 per cent disease incidence. *In vitro*, *Trichoderma asperellum* (TNAU Tv1) and Fusilazole 0.1% recorded 100 per cent mycelial inhibition, followed by *Bacillus subtilis* (Bbv57) with 93.85 per cent inhibition. In pot culture, Fusilazole 0.1% recorded the highest disease reduction (83.92%). Under field conditions, it was the most effective treatment, providing 44.12 per cent disease reduction, 13.9 t ha⁻¹ bulb yield and a B:C ratio of 1.89. *T. asperellum* also showed promising performance, with 38.0 per cent disease reduction, 12.7 t ha⁻¹ yield and a B:C ratio of 1.72. Overall, Fusilazole 0.1% and *T. asperellum* (TNAU Tv1) were identified as promising components of an integrated management programme for onion twister blight. Further field validation under diverse agro-climatic conditions is recommended.

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Conceptualization, K.Y, P.M, & A.K; Formal analysis; K.Y, P.M & J.M; Supervision and validation ; S.S, K.Y, A.K & J.M ;Language, S.S, R.N & D.J

Consent for publication

All authors consent the publication of the current article.

Competing interests

The authors declare no competing interests.

REFERENCES

1. Nasri S, Anoush M, Khatami N. 2012. Evaluation of analgesic and anti-inflammatory effects of fresh onion juice in experimental animals. *Afr J Tradit Complement Altern Med.* 6:1679–1684.
2. Anonymous. Indian Horticulture Database. 2015, 198.
3. Alberto RT. 2014 – Pathological response and biochemical changes in *Allium cepa* L. (bulb onions) infected with anthracnose-twister disease. *Plant Pathology and Quarantine* 4(1), 23–31. Doi: 10.5943/ppq/4/1/4
4. Chandel and Deepika, 2010. Recent advances in management and control of fusarium yellows in gladiolus species. *J. Fruit Ornament. Plant Res.* vol. 18(2) 2010: 361–380
5. Jamil, S. and M. Kumar. 2010. Evaluation of fungicides against phyllosphere mycoflora of foliage plants. *Biological Forum*, 2 (1): 56–59.
6. Manthesha HD, Mallikarjun Kenganal, Yenjerappa ST, Aswathanarayana DS and Vikas Kulkarni. Management of onion twister disease under field condition. 2022. *The Pharma Innovation Journal* 11(4): 551–555
7. Perez PM, Alberto RT 2020 – Chemical Management of Anthracnose-Twister (*Colletotrichum gloeosporioides* and *Fusarium fujikuroi*) Disease of Onion (*Allium cepa*) *Plant Pathology & Quarantine* 10(1), 198–216
8. Bhagwat R. G, Mehta B. P and Patil V. A. 2016. Evaluation of fungicides and biological agents for the management of mango anthracnose. *International Journal of Environmental & Agriculture Research.* 2 (4): 49–52.