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In-vitro Management of Wilt Disease of Sugarcane Through Optimization of Chemical Fungicides

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Wilt of sugarcane caused by *Fusarium sacchari*, is a soil and seed borne pathogen causing heavy losses in sugarcane production to grower. Chemical control method is an effective and highly adopted approach of eliminating disease causing organism. The present study was carried out to assess the efficacy of combination fungicides in vitro condition against *Fusarium sacchari* causing

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wilt in sugarcane. Three combination (systemic fungicides) and one contact fungicide Mastercop (Copper sulphate pentrahydrate 23.99% SC), Amistar-Top (Azoxystrobin 18.2 SC + Difenoconazole 11.4 SC), Shamir (Tebuconazole 6.7 + Captan 26.9SC), Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS), Dhanustin (Carbendazim 50% WP) were tested at three different concentration (5PPM, 15PPM and 25PPM) using by poisoned food technique on OMA medium. All the fungicides significantly inhibited mycelial growth of the fungus. Maximum 100% mycelial growth inhibition was recorded in **T_4** Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS) at all concentrations and minimum 69.25% mycelial growth inhibition was recorded in **T_1** Mastercop (Copper sulphate pentrahydrate 23.99% SC) and all other fungicides significantly inhibited the mycelial growth of the fungus, observation was recorded and mentioned in the result of the paper.

Keywords: Mycelial growth; wilt disease; chemical fungicides; waterlogging.

1. INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a perennial tall belong to the family Poaceae. The species *officinarum* is a richest source of sucrose that is accumulated in the stalk internodes of sugarcane as a juicy fiber. Sugarcane is extensively grown in all the tropical and sub-tropical regions of India as a major cash crop for the supply of raw material in the sugar industries for the production of sugar, khandsari, & gur. Sugarcane is cultivated in a large area of Uttar Pradesh. The area of cane in entire Uttar Pradesh is about 28.53 lakh hectares, whose productivity is 83.9 tons per hectare. Since Sugarcane is an annual crop, it has to go through all the seasons of the years, in which the problem of waterlogging in the terai areas after the rains become the main reason for the occurrence and spread of wilt disease in sugarcane [10,11]. Wilt is one of the earliest known diseases of sugarcane in India and was first reported by Butler (1906) from Bihar state. Wilt epidemics in India during the last century resulted in elimination of many commercial cultivars from cultivation (Kirtikar *et al*., 1972; Singh and Singh, 1974; Subba Raja and Natarajan, 1972). Later also very severe wilt incidences were noticed in South Gujarat and in different parts of Gangetic plains. Country-wide disease assessment revealed that wilt of 60% on Co 7717, 5-10% in CoJ 64, CoJ 79,CoS 767 and popular variety Co 0238 in Uttar Pradesh, severe wilt incidence in combination with red rot noticed on major varieties in Bihar, severe wilt incidence on Co 89003 and moderate wilt on Co 7717, CoS 8436 and CoS 88230 in Punjab, varying levels of wilt in most of the varieties in cultivation in South Gujarat, mild wilt on popular varieties in Maharashtra and in Madhya Pradesh (Agnihotri and Rao, 2002; Benício et al., 2003). Previous studies of Viswanathan *et al*. (2006) revealed that the disease intensity vary from trace to 75% in different states of India. Wilt in the cv. Co 7805, an elite variety in coastal Andhra Pradesh caused enormous loss to sugarcane production in the past two decades (Viswanathan, 2013; Ashwini et al., 2024). Butler and Khan (1913) for the first time described the disease in India in sugarcane under the term 'wilt' and noted *Cephalosporium sacchari* as the causal agent. The first author has witnessed such wilt infections in young crops in Gujarat and other places where the disease is epidemic. Here it was found that infected setts serve as the primary source for wilt development (Viswanathan, 2012, 2013).

1.1 External and Internal Symptoms of wilt Disease in Sugarcane

In the month of September-October, the symptom of the disease start appearing on the new leaves, first the leaves start turning yellow and chlorosis from the edges. It is a little difficult to identify the disease in the initial stages because these symptoms are similar to the symptoms of nutrient deficiency in the plants. The new life start drying from the edges as the infection increases. In case of severe infection, the entire plant dries up. When the infected sugarcane is torn from the middle, the inner parts appears hollow in with the mycelium of the fungus is seen growing, the infected sugarcane does not break easily when broken and gets flattened from the node (Nithya et al., 2024). Unlike the red rot disease, there is no alcohol-like odor in the infected sugarcane. The pores appear stuck in the middle. The yield of the infected sugarcane fields is very low due to which the farmers have to face a lot of economic losses. Sometimes, in case of severe infection, the entire crop dries up and gets destroyed and the farmer suffers 100% losses in economic yield, along with this the sugar layer decreases due to witch sugar mills suffer losses.

Fig. 1. External and internal symptoms of wilt disease in sugarcane at field condition

2. MATERIALS AND METHODS

The present study was conducted in the Laboratory of Plant Pathology, Sugarcane Research Institute, Shahjahanpur, during seasons 2024-25, evaluation of three combination and one contact fungicides namely Mastercop (Copper sulphate pentrahydrate 23.99% SC), Amistar-Top (Azoxystrobin 18.2 SC + Difenoconazole 11.4 SC), Shamir (Tebuconazole 6.7 + Captan 26.9SC) 7.1% + Propiconazole 11.9 SE), Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS) against *Fusarium spp*. causing wilt in sugarcane.

2.1 Concentration of Fungicide

Three combination (systemic fungicides) and one contact fungicide Mastercop (Copper sulphate pentrahydrate 23.99% SC), Amistar-Top (Azoxystrobin 18.2 SC + Difenoconazole 11.4 SC), Shamir (Tebuconazole 6.7 + Captan 26.9SC), Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS), Dhanustin (Carbendazim 50% WP) were used to test the efficacy of their potential against *Fusarium spp*. at the concentration levels of 5 PPM, 15 PPM, and 25 PPM. Fungicidal concentrations were prepared by adding measured quantity of active ingredient. All fungicides at different tested concentrations with three replications by using of poisoned food technique. Fungicide was obtained from registered pesticide dealers available in the local market.

2.2 Collection of Diseases Sample

Wilt disease infected sugarcane samples were collected from the farmer fields during the survey programs. The infected cane was identified on the basis of the above mentioned external and internal disease symptoms and the sample obtained from the field were brought to the Plant Pathology laboratory of UPCSR Shahjahanpur for further study.

2.3 Preparation of Culture Media

2.3.1 (A) OMA (Oat Meal Agar)

Oat meal powder 35.0 grams and Agar-Agar 15.0 grams was added in 1000 ml pure distilled water and shake in 2 minutes than this solution was heated up to boiling to dissolve the medium completely keep mixing till it becomes like jelly then filled in conical flask and sterilize by autoclaving at 15 LBS pressures at 121ºC temperature for 15 minutes was carried out for disinfection. Than cool to 40-45ºC and then finally it was mix well and pore into sterile petri plates.

2.3.2 (B) PDA (Potato Dextrose Agar)

PDA (39.0 grams) was mixed in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes, i.e. validated cycle. It was mixed well before dispensing in Petri plate. In specific work, when pH 3.5 is required, acidify the medium with sterile 10% tartaric acid. The amount of acid required for 100 ml. of sterile, cooled medium is approximately 1 ml. Do not heat the medium after addition of the acid.

2.4 Isolation of Pathogen (*Fusarium spp*.) from wilt Affected Cane

First of all, after peeling the upper surface of the infected cane with the help of a knife, the surface is cleaned with the help of spirit, after that the sugarcane is torn inside the laminar airflow platform and the part with intense infection where the mycelium is growing towards the healthy tissues is cut and taken for inoculation in the pre-prepared culture medium and the inoculated plate is incubated at 28 ºC temperature with 75% Relative humidity for 7 days.

Fig. 2. (A). Oat Meal Agar culture medium for isolation

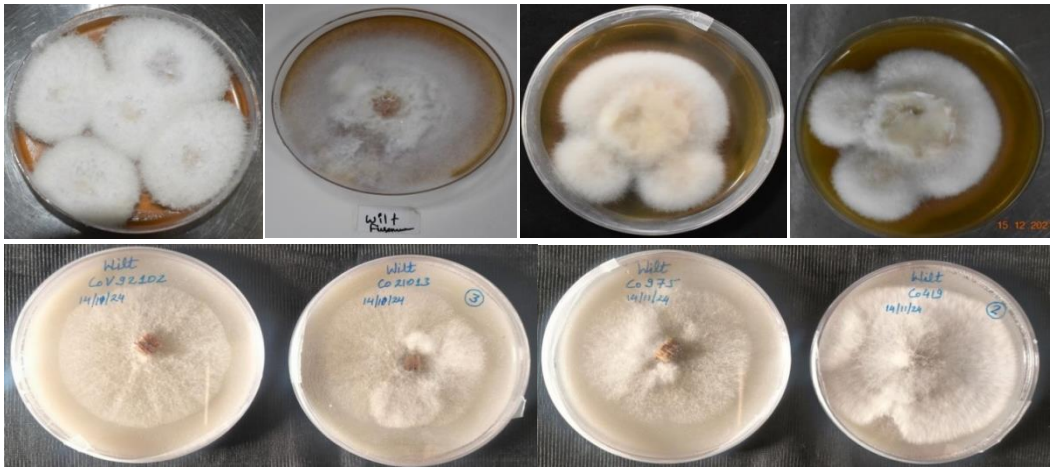

Fig. 3. Fungus colonies on PDA

2.5 The Poisoned Food Technique

The efficacy of Mastercop (Copper sulphate pentrahydrate 23.99% SC) Amistar-Top (Azoxystrobin 18.2 SC + Difenoconazole 11.4 SC), Shamir (Tebuconazole 6.7 + Captan 26.9SC), Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS) fungicide was taken for the study of the inhibiting the radial growth of *C. falcatum* through poisoned food technique on three different concentrations viz; 5, 15, 25 PPM. Each treatment was replicated three times. Oat meal agar was used and requisite concentration of each fungicide (a.i.g.L-^{1}) was added to get a required concentration. The fungicides were carefully mixed by stirring and about 20 ml poisoned medium was poured to each of the 90 mm petri dishes and allowed for solidification. Three culture plates (90 cm) were poured with OMA for each treatment. After the agar medium has solidified, 3 mm agar plugs containing mycelium of *Fusarium spp.,* were cut from the culture plates using sterilized cork borer and were placed in the center of each agar plate. Suitable control was maintained on OMA having no fungicide. These plates were incubated at 28 ± 2°C. The diameter of mycelium growth was recorded after 7 DAI (Days After Incubation). Corresponding controls were also maintained, simultaneously. Percent inhibition of *Fusarium spp.,* colonies in each treatment was recorded over the control.

The percent inhibition in growth due to various fungicidal treatments at different concentrations was computed as follows (Benicio *et al*., 2003).

$$\mathrm{PGI}\ \% = \frac{\mathrm{C} - \mathrm{T}}{\mathrm{C}} \times 100$$

(PGI = Percent growth inhibition, C = Colony diameter in control plate, T = Colony diameter in intersecting plate.

3. RESULTS AND DISCUSSION

All of the combination fungicides were found to exhibit mycilial inhibition that was noticeably better than the control. Among all the fungicides tested at three concentrations (5PPM, 15 PPM, 25PPM), maximum percent mycelial inhibition

was recoded in treatment T4; Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS) at all the three concentrations (100%) which was found significantly superior over the rest of the treatment followed by T5; Dhanustin (Carbendazim 50 % WP) at 5PPM (97.3%), 15PPM (99.0%), 25PPM (100.0%) percent mycelial growth inhibition. The least mycelial growth inhibition was recorded in Mastercop (Copper sulphate pentrahydrate 23.99% SC) at 5PPM concentration (61.11%) percent. Irrespective of concentration of combination fungicides tested, the treatment involving Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS) recorded maximum mean percent mycelial inhibition (100.0%) followed by Dhanustin (Carbendazim 50 % WP) (98.76%) and minimum average percent mycelial growth inhibition was recorded in Mastercop (Copper sulphate pentrahydrate 23.99% SC) (69.25) percent. Tested the efficacy of different fungicides in vitro conditions against fusarium wilt of sugarcane; Electron fungicides was very effective at its all concentration and Carbendazim gave the superior result at 25PPM concentration with 100 percent mycelial growth inhibition.

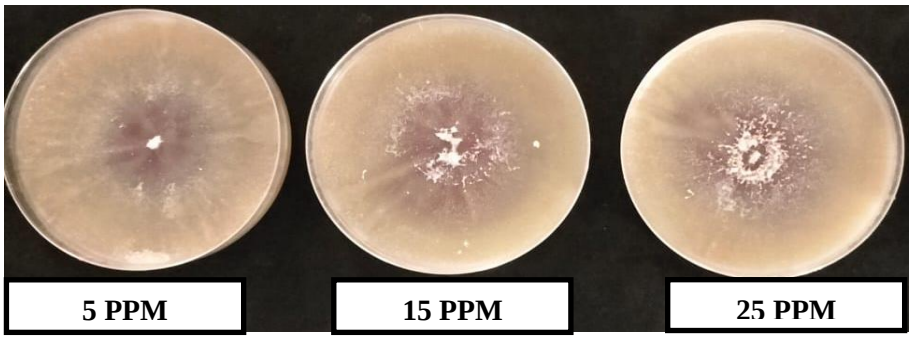

Fig. 4. Mycelial growth inhibition of (T_1) Mastercop (Copper sulphate pentrahydrate 23.99% SC)

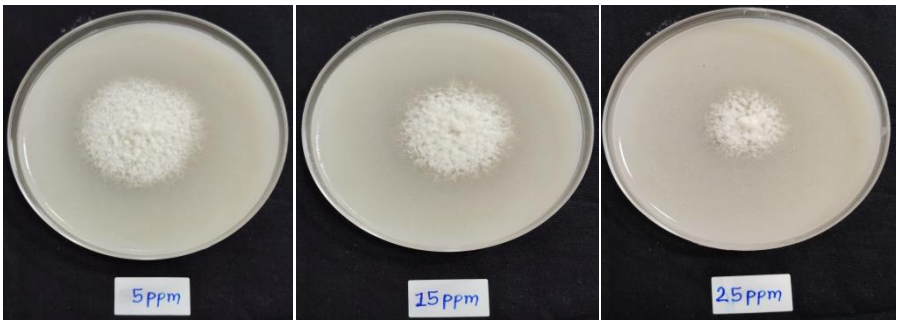

Fig. 5. Mycelial growth inhibition of (T_2) Amistar-Top (Azoxystrobin 18.2 SC + Difenoconazole 11.4 SC)

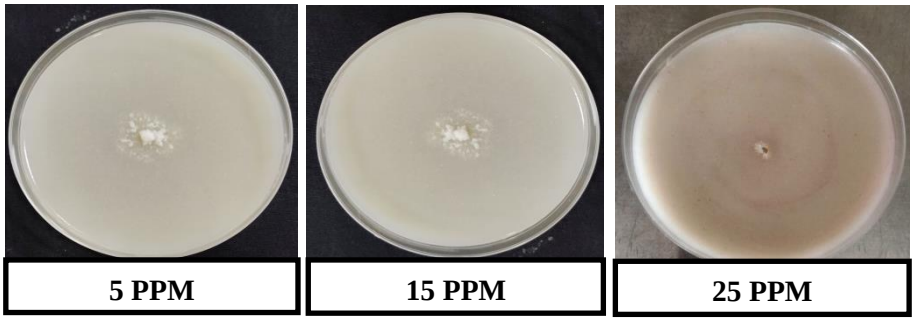

Fig. 6. Mycelial growth inhibition of (T_3) Shamir (Tebuconazole 6.7% + Captan 26.9 % SC)

Table 1. In vitro evaluation of combination fungicides against wilt of sugarcane.

Sr. No.	Treatment with three Concentration	Trade Name and Formulation	Percent inhibition of mycelium growth			Ave. Dimension of fungal growth	Mean % Inhibition
			Concentration (ppm)				
			5	15	25		
1.	T_1	Mastercop (Copper sulphate pentrahydrate 23.99% SC)	61.11	68.88	77.77	1.02	69.25
2.	T_2	Amistar-Top (Azoxystrobin 18.2 SC + Difenoconazole 11.4 SC)	68.88	77.77	86.66	1.54	77.77
3.	T_3	Shamir (Tebuconazole 6.7% + Captan 26.9 % SC)	63.2	71.01	78.4	0.61	70.87
4.	T_4	Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS)	100.0	100.0	100.0	0.00	100.0
5.	T_5	Dhanustin (Carbendazim 50 % WP)	97.3	99.0	100.0	0.12	98.76
9.	T_6	Control	_	_	_	100.0	0.0
	C.V	**5.063**					
	C.D at 1% and 5% Level	**11.55**, **7.94**					

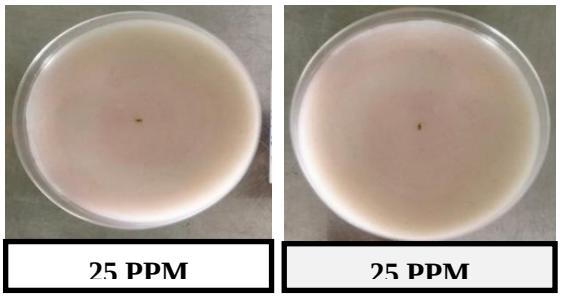

Fig. 7. Mycelial growth inhibition of (T_4) Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS)

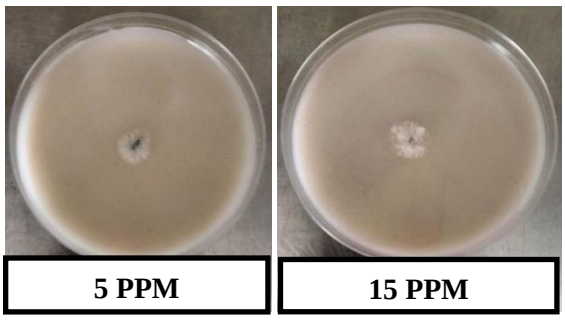

Fig. 8. Mycelial growth inhibition of (T_4) Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS)

4. CONCLUSION

Among combination fungicides tested, treatment **(T_4)** Electron (Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS) recorded highest inhibition of mycelial growth (100.0%) and least mycelial growth inhibition was observed **(T_1)** Mastercop (Copper sulphate pentrahydrate 23.99% SC) (69.25%). In all the fungicides, inhibition of mycelial growth increased with increase in concentration.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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