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In vitro Evaluation of Fungicides against *Fusarium sacchari* Causing Pokkah Boeng Disease of Sugarcane

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ABSTRACT

Pokkah boeng disease caused by *Fusarium sacchari*, is an air borne pathogen causing heavy losses in sugarcane production. Chemical control method is an effective and highly adopted approach of eliminating disease causing organisms. The present study was carried out to assess the efficacy of fungicides *in vitro* against *Fusarium sacchari* causing Pokkah boeng disease of sugarcane. The fungicides, *viz.,* carbendazim, propiconazole, tebuconazole, difenoconazole, azoxystrobin, azoxystrobin + tebuconazole, trifloxystrobin + tebuconazole, azoxystrobin + difenoconazole, copper oxy chloride and mancozeb, were tested at two different concentrations (1000 ppm and 500 ppm) using poisoned food technique on potato dextrose agar medium. All the fungicides inhibited the fungal growth significantly, among which carbendazim, difenoconazole, azoxystrobin + difenoconazole, tebuconazole, propiconazole and trifloxystrobin + tebuconazole were highly effective at both the concentrations tested with 100% inhibition in mycelial growth followed by azoxystrobin + tebuconazole, copper oxy chloride and azoxystrobin at both concentrations tested. Mancozeb showed least inhibition *i.e.*, 22.47% at 1000 ppm and 2.58% at 500 ppm. The chemicals exhibited increased tendency of inhibition with increased concentration.

Keywords: *Fungicides, Fusarium sacchari and Poisoned food technique.*

Sugarcane (*Saccharum officinarum* L.) is one of the most important cash crops in India. Globally, Brazil is the largest producer of sugarcane followed by India, China, Thailand, Pakistan and Mexico (Sarwar *et al*., 2010). Sugarcane belongs to the genus *Saccharum* which is composed of hybrids (Price, 1965; Arceneaux, 1967) derived from *Saccharum officinarum* (noble clones), *S. sinense* (Chinese clones), *S. barberi* (North Indian clones) and *S. spontaneum*. In India, sugarcane is grown in 4.55 M ha area with annual production of 353.84 million tonnes and productivity around 77.75 tonnes ha-1. (www.indiaagristat.com, 2019-20). Major sugarcane growing areas in India are Uttar Pradesh which accounts for 178.42 million tonnes production followed by Maharashtra, Karnataka, Bihar, Tamil Nadu, Panjab, Haryana, Gujarat and Andhra Pradesh. In Andhra Pradesh, sugarcane occupies an area of 0.99 lakh ha, giving a total production of 65.5 lakh tonnes and productivity of 76.14 tonnes ha^{-1} (www.indiaagristat.com, 2019-20).

One of the emerging diseases affecting sugarcane and sugar production is Pokkah boeng disease caused by *Fusarium* species complex, a destructive fungal disease in sugarcane-growing regions. This disease is associated with several diseases of sugarcane such as sett rot, root rot, and wilt (Waraitch *et al.,* 1982). The pathogen is

transmitted by air currents, and airborne conidia colonize the leaves, flowers and stems of the plant. Pokkah boeng disease is considered as a minor foliar disease earlier and is now emerging as a major disease, causing substantial losses in cane weight, length, girth, total juice and total sugars (Singh *et al.,* 2006).

In India, *Fusarium sacchari*, *F. proliferatum* and *F. moniliforme* var. *subglutinans* were found associated with Pokkah boeng disease (Arya *et al.,* 2017). This pathogen is capable of surviving in the soils as chlamydospores that can remain viable for several years (Erskine and Bayaa, 1996) and can attack the next crop in the field even when the crop is sown after a long interval. This might be the reason for its devastating effect in recent times.

The management practices for this disease include the use of resistant varieties, spraying of fungicides and use of biocontrol agents. Each control method has got its significance but none is able to work solely. Popular cultivars of sugarcane like Co 0238, CoSe 92423 and CoSe 95422 were found highly susceptible to the disease. Several cultivars have shown field resistance against Pokkah boeng disease of sugarcane (Anuradha *et al*., 2018). According to Bendre and Barhate (1998), the management strategies should include modified cultural practices, resistant varieties, beneficial biocontrol agents and minimum use of chemicals. Chemical control method is the widely applicable and most preferred method for killing the pathogens.

Fungicides usage has been proved to be the quick and effective control strategy (Maitlo *et al*., 2014; Jyothsna *et al.,* 2018; Amulya *et al.,* 2018). Various chemicals are available for control of *Fusarium* pathogen. Finding the most effective chemical with reliable dose against the pathogen is the need of the hour for those willing to invest their time and capital in sugarcane cultivation. Several fungicides were found effective for the management of Pokkah boeng disease. Spraying of fungicides like carbendazim, copper oxychloride, mancozeb, propiconazole and difenoconazole on first appearance of disease was found effective for disease management (Vishwakarma *et al*., 2013; Sharma and Arun, 2015; Jagadeeshwar *et al.,* 2014). This study was focused towards finding the effectiveness and optimum doses of fungicides that are popularly used in disease control. Therefore, the present study was carried out to compare *in vitro* efficacy of various fungicides against *F. sacchari*, a Pokkah boeng causing pathogen of sugarcane.

MATERIALS AND METHODS

The experiment was conducted during 2021 at the Department of Plant Pathology, Agricultural College, Bapatla. The study was designed with ten treatments and three replications. *In vitro* efficacy of different fungicides against *Fusarium sacchari* was studied by Poisoned Food Technique (Vincent, 1947). The fungicides *viz.*, carbendazim, propiconazole, tebuconazole, difenoconazole, azoxystrobin, azoxystrobin + tebuconazole, trifloxystrobin + tebuconazole, azoxystrobin + difenoconazole, copper oxychloride and mancozeb were evaluated against the test fungus at the concentrations of 500 and 1000 ppm along with check (Table 1).

Ten ml of stock solution of 1,00,000 ppm concentration of each fungicide was prepared in the distilled water in test tubes. Calculated quantity of the solution was added using micropipette into 150 ml flask containing 60 ml of the sterilized molten PDA, so as to get final required concentrations of 500 and 1000 ppm. The medium was mixed thoroughly before plating. Such poisoned medium was poured separately into Petri plates. Non toxicated media poured into Petri plates was kept as check. After solidification of

the media, a 5 mm mycelial disc of seven day old culture of the pathogen was cut with sterilized cork borer and placed at the center of each Petri plates. The Petri plates were incubated at 26-28°C. After 10 days of incubation, the radial growth was measured from the treatments when the growth of the control plates completely covered the plates (9 cm diameter). The per cent inhibition in growth was determined using the following formula:

$$I = \frac{C - T}{C} \times 100$$

Here,

I = per cent inhibition,

C = growth of the fungus in non-poisoned food medium and,

T = growth of the fungus in poisoned food medium.

RESULT AND DISCUSSION

All the test fungicides showed significant difference in their efficacy for inhibiting radial growth of *Fusarium sacchari* compared to control (Table 2, Figure 1, Plate 1). The highest per cent inhibition (100%) was recorded in carbendazim, difenoconazole, azoxystrobin + difenoconazole, tebuconazole, propiconazole and trifloxystrobin + tebuconazole at both the concentrations *i.e.*, 500 and 1000 ppm, where diameter of fungal colony observed was zero cm. The least per cent inhibition was observed in case of mancozeb at both the concentrations *i.e.*, 500 and 1000 ppm. It has been noticed that effectiveness of mancozeb at 500 ppm concentration was very less (2.58 per cent inhibition) which allowed the pathogen to grow 8.67 cm diameter of the Petri plate when compared with 1000 ppm concentration at which 22.47 per cent inhibition was recorded with 6.9 cm diameter growth of *Fusarium sacchari*.

At 1000 ppm concentration, azoxystrobin + tebuconazole, copper oxy chloride followed by azoxystrobin restricted the colony growth to 1.80, 3.77 and 6.13 cm diameter with 79.78, 57.64 and 31.12 per cent inhibition, respectively. At 500 ppm concentration azoxystrobin + tebuconazole, azoxystrobin and copper oxy chloride restricted the colony growth to 2.57, 6.83 and 8.40 cm diameter colony growth with 71.12, 23.26 and 5.62 per cent inhibition, respectively. They showed high efficacy at 1000 ppm concentration comparing to 500 ppm concentration.

Data indicated complete inhibition of mycelial growth in carbendazim, difenoconazole, azoxystrobin + difenoconazole, tebuconazole, propiconazole and trifloxystrobin + tebuconazole treated media at 500 ppm and 1000 ppm and they were statistically on par with each other regarding their efficacy against the pathogen at both concentrations and showed significant variation with mancozeb, azoxystrobin, copper oxy chloride and azoxystrobin + tebuconazole. So, the former chemicals were proved to be the best ones to control the pathogen causing Pokkah boeng disease of sugarcane.

At 1000 ppm concentration, dithiocarbamate fungicide like mancozeb (22.47 per cent inhibition) was less effective compared to strobilurin compounds like azoxystrobin (31.12 per cent inhibition). Further, the efficacy of azoxystrobin was less when compared to copper oxy chloride (57.64 per cent inhibition). Azoxystrobin in combination with tebuconazole gave remarkable results (79.78 per cent inhibition).

Even at 500 ppm concentration, mancozeb (2.58 per cent inhibition) and copper oxy chloride (5.62 per cent inhibition) were found significantly less effective compared to their efficacy at higher concentration (1000 ppm). Azoxystrobin (23.26 per cent inhibition) was found ineffective when compared with azoxystrobin + tebuconazole (71.12 per cent

Table 1. List of fungicides evaluated against *Fusarium sacchari in vitro*

S. No.	Treatments	Common Name	Trade Name	Formulation
1	T1	Carbendazim	Kemistan	50% WP
2	T2	Copper oxychloride	Viva Copper	50% WP
3	T3	Azoxystrobin + Tebuconazole	Custodia	11.00% + 18.3% SC
4	T4	Difenoconazole	Score	25% EC
5	T5	Mancozeb	Indofil M-45	75% WP
6	T6	Azoxystrobin + Difenoconazole	Amistar top	18.2% + 11.4% SC
7	T7	Azoxystrobin	Amistar	25% EC
8	T8	Tebuconazole	Folicur	25.9% EC
9	T9	Propiconazole	Bumper	25% EC
10	T10	Trifloxystrobin + Tebuconazole	Nativo	50% + 25% WG

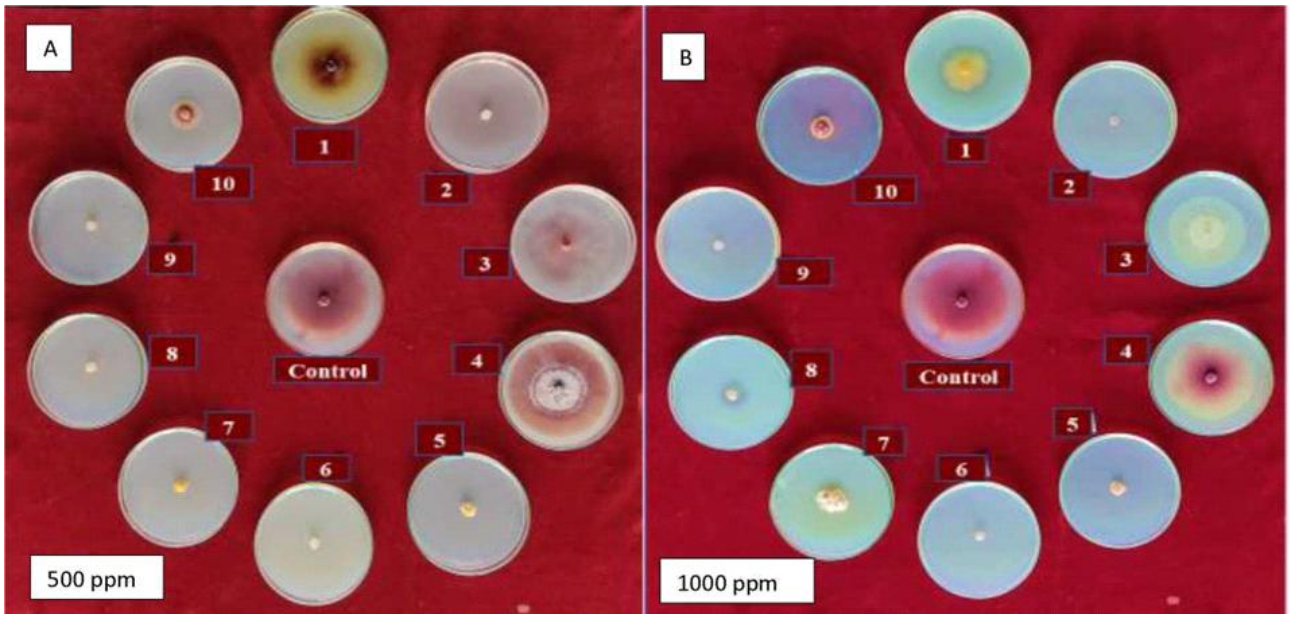

Plate 1. Inhibitory effect of fungicides on radial growth of *Fusarium sacchari in vitro* (A) Effect of fungicides at 500 ppm concentration (B) Effect of fungicides at 1000 ppm concentration. Where numbers depicting different fungicides 1. Copper oxy chloride 2. Propiconazole 3. Azoxystrobin 4. Mancozeb 5. Difenoconazole 6. Carbendazim 7. Azoxystrobin + difenoconazole 8. Tebuconazole 9. Tebuconazole + trifloxystrobin 10. Azoxystrobin + tebuconazole

Table 2. Efficacy of fungicides on per cent inhibition of mycelial growth of *Fusarium sacchari.*

S. No.	Fungicides	Colony diameter 8 DAI (mm)		Mean mycelial growth		Per cent inhibition over control		Mean% inhibition
		500 ppm	1000 ppm			500 ppm	1000 ppm	
1	Carbendazim	0.00 *(1.00) [e]	0.00 (1.00) [e]	0.00 (1.00)		100.00	100.00	100.00
2	Copper oxy chloride	8.40 (3.07) [b]	3.77 (2.18) [c]	6.08 (2.62)		5.62	57.64	31.63
3	Azoxystrobin +Tebuconazole	2.57 (1.89) [d]	1.80 (1.67) [d]	2.18 (1.78)		71.12	79.78	75.45
4	Difenoconazole	0.00 (1.00) [e]	0.00 (1.00) [e]	0.00 (1.00)		100.00	100.00	100.00
5	Mancozeb	8.67 (3.11) [a]	6.90 (2.81) [a]	7.78 (2.96)		2.58	22.47	12.53
6	Azoxystrobin + Difenoconazole	0.00 (1.00) [e]	0.00 (1.00) [e]	0.00 (1.00)		100.00	100.00	100.00
7	Azoxystrobin	6.83 (2.80) [c]	6.13 (2.67) [b]	6.48 (2.73)		23.26	31.12	27.19
8	Tebuconazole	0.00 (1.00) [e]	0.00 (1.00) [e]	0.00 (1.00)		100.00	100.00	100.00
9	Propiconazole	0.00 (1.00) [e]	0.00 (1.00) [e]	0.00 (1.00)		100.00	100.00	100.00
10	Trifloxystrobin + Tebuconazole	0.00 (1.00) [e]	0.00 (1.00) [e]	0.00 (1.00)		100.00	100.00	100.00
Mean		2.64 (1.69)	1.86 (1.73)					
Control		8.9 (3.1464) (C)						
		Fungicides (T)	Concentration	T X C	C Vs T			
SEm ±		0.0069	0.0069	0.0156	0.012			
C.D.(P<0.05)		0.01	0.01	0.03	0.03			
CV%		1.14						

Square root transformed values are provided in parenthesis.

inhibition) combination but better than mancozeb and COC. The present study indicated inefficacy of mancozeb fungicides against *F. sacchari in vitro*.

Tebuconazole, alone showed 100% inhibition at both the concentrations but its combination with azoxystrobin showed reduced efficacy (79.78 and 71.12 per cent inhibition at 500 and 1000 ppm, respectively) by restricting the growth of the pathogen to 2.57 cm and 1.80 cm diameter at 500 and 1000 ppm concentrations, respectively. This may be due to less concentrated formulation (Azoxystrobin + Tebuconazole: 11.00% + 18.3% SC; Tebuconazole: 25.9% EC). Whereas, azoxystrobin had less efficacy when used alone against the pathogen *in vitro*.

Fungicides at their higher (1000 ppm) concentrations showed better efficacy in controlling the growth of the pathogen when compared with their lower (500 ppm) concentration against the same pathogen.

Similar results were observed by Chauhan (2014) regarding propiconazole and difenoconazole which have totally inhibited the growth of *Fusarium moniliforme* f. sp. *subglutinans* even at 5 ppm. Jain *et al.* (2014) demonstrated the potential of systemic fungicides (carbendazim, hexaconazole, tebuconazole and thiophanate) against *F. moniliforme* compared to non-systemic fungicides such as mancozeb, propineb and thiram.

The results obtained showed deviation from Sharma and Kumar (2015) who reported copper oxy chloride as highly efficacious compared to carbendazim at 1000 ppm against *F. moniliforme* with 98 per cent and 91 per cent inhibition over control, respectively. But in our studies carbendazim showed 100% inhibition at 500 and 1000 ppm whereas, COC was effective at 1000 ppm that to with 57.64% inhibition only.

Wani *et al*. (2011) found that all the systemic fungicides *viz.*, carbendazim. bitertanol, myclobutanil, hexaconazole, non systemic fungicides like mancozeb, captan and zineb at different concentrations significantly inhibited the mycelial growth of *F. oxysporum*.

CONCLUSION

In the present study, laboratory testing of ten fungicides at two different concentrations (500 and 1000 ppm) by poisoned food technique revealed that all the ten fungicides showed effectiveness in decreasing the fungal growth at higher concentration of 1000 ppm. Carbendazim, difenoconazole, azoxystrobin + difenoconazole, tebuconazole, propiconazole and trifloxystrobin + tebuconazole were proved to be the best among the tested fungicides which completely inhibited the fungal growth at both the concentrations. Azoxystrobin + tebuconazole, copper oxy chloride and azoxystrobin were moderately effective while mancozeb ranked last among these fungicides. This information provides a choice to the sugarcane growers to choose chemicals against the Pokkah boeng pathogen and the researchers to consider some of these fungicides for field evaluation against pokkah boeng disease.

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