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In vitro and *In vivo* efficacy of different chemicals against Bacterial leaf blight disease of rice caused by *Xanthomonas oryzae* Pv. *oryzae*

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Abstract
Rice (*Oryza sativa* L.) belongs to family Poaceae (Gramineae) and widely cultivated in the India. The maximum losses of rice crop by Bacterial leaf blight stands one of the important disease of rice caused by *Xanthomonas oryzae* Pv. *oryzae* and which reduces the yield upto 50% in the world. In India yield losses due to this disease is up to 81.3%. In present investigation, different chemicals were tested under *in vitro* and *in vivo* condition against Bacterial leaf blight disease of rice causing by *Xanthomonas oryzae* pv. *oryzae*. Combination of [Azoxystrobin 25% + Difenoconazole 12.5%] @ 300 ppm + Streptocycline @ 200 ppm was found most effective with inhibition 31.62% and was at par with [Mancozeb (12%) + Carbendazim (63%)] @ 500 ppm + Streptocycline @ 200 ppm 30.48% inhibition against *Xanthomonas oryzae* pv. *oryzae*. Fungicide alone tested at different concentration and found that [Azoxystrobin 25% + Difenoconazole 12.5%] @ 300 ppm was found most effective with inhibition 21.66% and was at par with [Mancozeb (12%) + Carbendazim (63%)] @ 500 ppm *i.e*. 19.33% inhibition, whereas Streptocycline @ 200 ppm showed 23.49% inhibition against Bacterial leaf blight disease of rice *in vitro* condition. Under *in vivo* condition five fungicides and two antibiotics were tested and found that the combination of Copper oxychloride @ 0.3% + Streptocycline @ 0.005% showed minimum disease incidence with 39.64%. Therefore, it could be useful to the farmers to manage the Bacterial leaf blight disease of rice by application of Copper oxychloride @ 0.3% + Streptocycline @ 0.005%.

Keywords: *In vitro*, *In vivo*, rice, bacterial leaf blight, *Xanthomonas oryzae* pv. *oryzae*

Introduction
Rice (*Oryza sativa* L.) is an important staple cereal crop and growing all over the world. More than half rice produced and consumed by Asian country in which 150.8 million hectares of rice plant annually and approximately 95% of rice consuming people belongs to Asian country (Singh *et al*., 2015) [11]. China and India both are major rice producing country which covers 49.1%. Rice is attacked by many fungal, bacterial, viral and nematodes pathogens. Among the bacterial diseases, bacterial leaf blight is considered as a major treated to paddy production because of its wide spread, distribution and its destructiveness under favorable conditions. Bacterial leaf blight disease caused by *Xanthomonas oryzae* pv. *oryzae* is one of most destructive disease of rice and oldest diseases recorded in the world and it was first reported from Fukuoka area of Japan in 1884. Bacterial leaf blight disease occurs in almost all the paddy growing region in the state, it is considered to be a major constraint for low rice productivity in India. In world, due to this disease yield loss was estimated approx 50% (Kulkarni and Jahagirdar 2011) [7] and in India 81.3% (Prasad *et al*. 2018, Swati *et al*. 2015) [9]. The earlier studies have identified some chemicals and antibiotics with relative against the disease. However, effective control of this disease has not been recorded. The present investigation was to bring together comprehensive update to the research on the bacterial disease of rice and the management strategies in Indian. Henceforth, the present work was undertaken to evaluated the effectiveness of some antibiotics and fungicides and in combination for the control of bacterial leaf blight disease of rice.

Material and Methods
Collection of Bacterial leaf blight samples
The present investigstion was conducted in PG laboratory of department of Plant Pathology and at Bihar Agricultural research farm of Bihar Agricultural University (BAU), Sabour, Bhagalpur (Bihar) during Kharif 2018. The diseased leaves of rice Rajendra Shweta showing typical bacterial leaf blight (BLB) were collected in brown paper bags.

Isolation of *Xanthomonas oryzae* pv. *oryzae*

Isolation of *Xanthomonas oryzae* pv. *oryzae* was carried out using infected leaves of rice plant collected from Bihar Agricultural Research farm of BAU Sabour, Bhagalpur. The typical showing symptoms of bacterial leaf blight and oozing from the cut section of leaves were used for isolation of bacterium. The diseased portion along with health portion was cut into 0.5-1 cm pieces. Disease pieces were sterilized by surface disinfected in 1% sodium hypochlorite solution for 30 seconds. After surface sterilization the disease pieces were washed three times with sterilized distilled water in aseptic condition. The diseased pieces were then suspended in a test containing 3 ml of sterilized distilled water and squeezed gently with sterilized scalpel. When the water became slightly turbid due to oozing of bacterial cells, the suspension was serially diluted up to 10^3 dilutions in 9 ml sterile water blank. This suspension was streaked n nutrient agar media with the help of sterilized wire loop. The inoculated plates were incubated at room temperature 27±2°C for 48 hours. After the inoculation periods, observation was made for the development of well separated, typical, light yellow colored bacterial colonies resembling *Xoo*.

In vitro* evaluation of different chemicals against *Xanthomonas oryzae* pv. *oryzae

In the present investigation, four commonly available chemicals were taken *viz*. three fungicides, Tebuconazole 50% + Trifloxystrobin 25%, Azoxystrobin 25% + Difenoconazole 12.5%, Mancozeb (12%) + Carbendazim (63%) and one antibiotic *viz*. Streptocycline were evaluated against *Xanthomonas oryzae* pv. *oryzae*. All the chemicals were evaluated at three different concentration *i.e*. 100 ppm, 300 ppm, 500 ppm except Azoxystrobin 25% + Difenoconazole 12.5% which was tested at concentration 100 ppm, 200 ppm, 300 ppm. In another experiment, single concentration of streptocycline @ 200 ppm with different concentration of different chemicals was tested. Two days old culture of *Xanthomonas oryzae* pv. *oryzae* was spread over the solidified nutrient agar media. The filter paper disc (6 mm diameter) were soaked in the desired concentration for five minutes and transfer to surface of medium seeded with bacterial culture in petri plate (90 mm). Five replication of each treatments were maintained. The plate without any chemical was taken as control. These inoculated petri plates were kept in the refrigerator at 4^0C for 4 hours. These petri plates were incubated at 27°C for 48 hours, zone of inhibition were clearly observed and inhibition zone in each petri plates was measured in terms of diameter (mm) and results analyzed statistically.

In vivo* evaluation of different chemicals against *Xanthomonas oryzae* pv. *oryzae Seven commonly available chemicals were taken in which five fungicides *viz*. (Tebuconazole 50% + Trifloxystrobin 25%) @ 0.02%, (Azoxystrobin 25% + Difenoconazole 12.5%) @ 0.03%, [Mancozeb (12%) + Carbendazim (63%)] @ 0.15%], Copper oxychloride @ 0.3%, Copper hydroxide @ 0.3% and two was antibiotics *viz.* Streptocycline @ 0.005%, Oxytetracycline @ 0.005% and combination of these chemicals and antibiotics were evaluated against *Xanthomonas oryzae* pv. *oryzae* in field condition.

The trails were laid out in Randomized Block Design (RBD) with three replications using Rajendra Shweta variety of rice. The Rajendra Shweta plant were inoculated at tillering stage. For inoculation, the tip of 4-6 leaves/plant was clipped with scissor dipped in the bacterium suspension (10^8 cells/ml). After inoculation, the chemicals alone and their combination of chemicals were sprayed on rice plant. First chemicals spray was done at 1st appearance of bacterial leaf blight symptoms and 2nd after 10 days of 1st spray.

Results and Discussion

In vitro* effect of combination of different chemicals on *Xanthomonas oryzae* pv. *oryzae

Various chemicals (Table 1 and Plate 1) with tree levels of concentration were evaluated against *Xanthomonas oryzae* pv. *oryzae* in *in vitro* condition. The maximum mean radial bacterial colony growth (90 mm) was observed in [Tebuconazole 50% + Trifloxystrobin 25%] @ 100 ppm and it was followed by [Mancozeb (12%) + Carbendazim (63%)] @ 100 ppm (83.35 mm). The least was observed in [Azoxystrobin 25% + Difenoconazole 12.5%] @ 300 ppm + Streptocycline @ 200 ppm i.e. 61.54 mm followed by [Mancozeb (12%) + Carbendazim (63%)] @ 500 ppm + Streptocycline @ 200 ppm i.e. 62.62 mm. Among the combination of chemicals, combination of [Azoxystrobin 25% + Difenoconazole 12.5%] @ 300 ppm + Streptocycline @ 200 ppm was found most effective with inhibition 31.62% and was at par with [Mancozeb (12%) + Carbendazim (63%)] @ 500 ppm + Streptocycline @ 200 ppm 30.48% inhibition. The least effective combination was [Mancozeb (12%) + Carbendazim (63%)] @ 100 ppm + Streptocycline @ 200 ppm which showed 23.47% zone of inhibition against *Xanthomonas oryzae* pv. *oryzae*. Many reports have been published which showed inhibitory effect of different chemicals on *Xoo* in laboratory conditions. Prasad *et al*. (2018) [9] repored that combination of Streptocycline @ 0.03% + Carbendazim @ 0.15% was found most effective with the maximum inhibition 24.42 mm against bacterial leaf blight under *in vitro* condition.

Effect of all the three chemicals alone, without antibiotics [Azoxystrobin 25% + Difenoconazole 12.5%] @ 300 ppm was found most effective with inhibition 21.66% and found was at par with [Mancozeb (12%) + Carbendazim (63%)] @ 500 ppm i.e. 19.33% inhibition. It was observed that in [Tebuconazole 50% + Trifloxystrobin 25%] @ 100 ppm has no effect against *Xanthomonas oryzae* pv. *oryzae* bacteria. Similar reports of inhibition zone were reported by many researchers. Swati *et al*. (2015) also studied and recorded that Azoxystrobin 25 SC @ 1.0 ml/l was found highly effective against *Xanthomonas oryzae* pv. *oryzae* i.e. 20-25 mm inhibition and in case of Carbendazim 50 WP @ 1.0 g/l i.e. 15-20 mm inhibition. *In vitro* effect against *Xanthomonas axonopodis* pv. *citri* causing bacterial canker of kagzi lime 10.11 mm, 20.75 mm and 20.28 mm inhibition in Carbendazim @ 500 ppm, Mancozeb @ 500 ppm and Streptocycline @ 250 ppm respectively (Jadhav *et al*. 2018) [4]. Kamble *et al*. (2017) [6] studied and recorded that Mancozeb @ 500 ppm was highly effective i.e. 16.00 mm inhibition and 10.2 mm inhibition in case of Streptocycline @ 100 ppm against *Xanthomonas campestris* pv. *viticola* causing bacterial leaf spot of grapes.

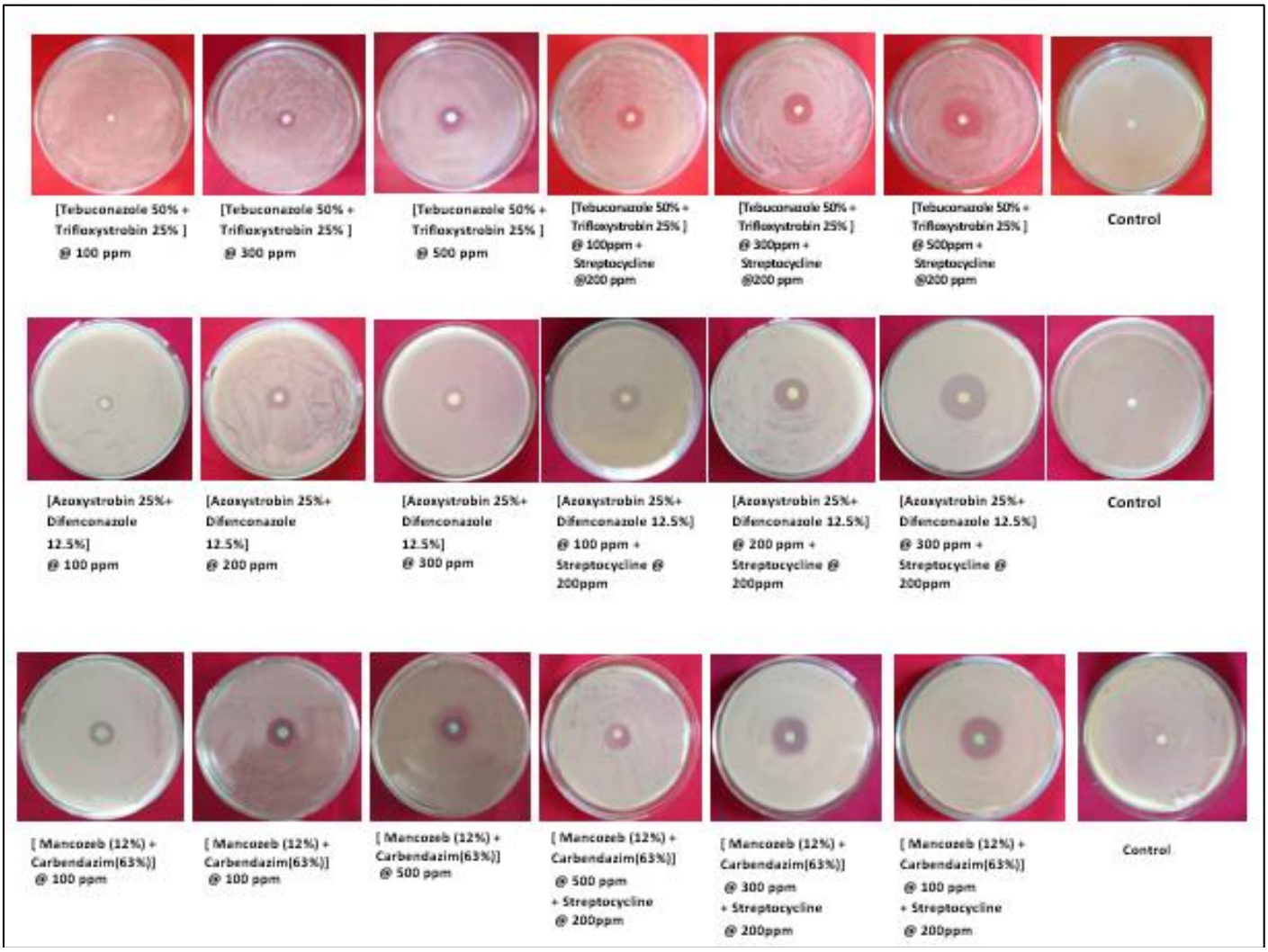

Plate 1: *In vitro* evaluation of different chemicals on *Xanthomonas oryzae* pv. *oryzae*.

Application of Streptocycline @ 200 ppm alone showed 23.49% inhibition whereas no inhibition effect was found in control. Similar results were found by Prasad *et al*. (2018) [9] who reported that Streptocycline @ 0.03% was highly effective antibiotics with 23.67 mm inhibition and streptomycin sulphate @ 200 ppm recorded 12.33 mm inhibition against *Xoo.* Meena *et al*. (2017) [8] also reported that Streptocycline @ 200 ppm showed 22.15 mm inhibiton against *Xanthomonas axonopodis punicae* causing pomegranate bacterial leaf blight. Streptocycline @ 200 ppm inhibit growth of *Xanthomonas axonopodis punicae* pathogen causing bacterial blight of pomegranate and found 1.88 cm inhibition (Ashish *et al*. 2016) [1].

Table 1: *In vitro* effect of different of different chemicals on *Xanthomonas oryzae* pv. *Oryzae*

S. No	Treatments	Mean radial bacterial colony Growth (mm)	Inhibition (%)
T_1	[Tebuconazole 50% + Trifloxystrobin 25%]@ 100 ppm	90.00 (64.16)*	0.00 (0.0)
T_2	[Tebuconazole 50% + Trifloxystrobin 25%]@ 300 ppm	82.64 (55.73)	9.95 (5.71)
T_3	[Tebuconazole 50% + Trifloxystrobin 25%]@ 500 ppm	78.04 (51.29)	13.28 (7.63)
T_4	[Tebuconazole 50% + Trifloxystrobin 25%]@ 100 ppm + Streptocycline @ 200 ppm	67.84 (42.71)	24.62 (14.25)
T_5	[Tebuconazole 50% + Trifloxystrobin 25%]@ 300 ppm + Streptocycline @ 200 ppm	65.52 (40.93)	27.20 (15.78)
T_6	[Tebuconazole 50% + Trifloxystrobin 25%]@ 500 ppm + Streptocycline @ 200 ppm	63.02 (39.06)	29.97 (17.44)
T_7	[Azoxystrobin 25% + Difenoconazole 12.5%]@ 100 ppm	80.30 (53.42)	10.77 (6.18)
T_8	[Azoxystrobin 25% + Difenoconazole 12.5%]@ 200 ppm	75.30 (48.85)	16.33 (9.40)
T_9	[Azoxystrobin 25% + Difenoconazole 12.5%]@ 300 ppm	70.50 (44.83)	21.66 (12.51)
T_{10}	[Azoxystrobin 25% + Difenoconazole 12.5%]@ 100 ppm + Streptocycline @ 200 ppm	67.64 (42.56)	24.84 (14.38)
T_{11}	[Azoxystrobin 25% + Difenoconazole 12.5%]@ 200 ppm + Streptocycline @ 200 ppm	64.32 (40.03)	28.53 (14.38)
T_{12}	[Azoxystrobin 25% + Difenoconazole 12.5%]@ 300 ppm + Streptocycline @200 ppm	61.54 (37.98)	31.62 (18.43)
T_{13}	[Mancozeb (12%) + Carbendazim (63%)] @ 100 ppm	83.35 (56.46)	7.37 (4.23)
T_{14}	[Mancozeb (12%) + Carbendazim (63%)] @300 ppm	81.94 (55.02)	8.95 (5.14)
T_{15}	[Mancozeb (12%) + Carbendazim (63%)] @ 500 ppm	72.60 (46.55)	19.33 (11.14)
T_{16}	[Mancozeb (12%) + Carbendazim (63%)] @ 100 ppm + Streptocycline @ 200 ppm	68.86 (43.52)	23.47 (13.57)
T_{17}	[Mancozeb (12%) + Carbendazim (63%)] @ 300 ppm + Streptocycline @ 200 ppm	65.98 (41.28)	26.69 (15.48)
T_{18}	[Mancozeb (12%) + Carbendazim (63%)] @ 500 ppm + Streptocycline @ 200 ppm	62.62 (38.77)	30.48 (17.74)
T_{19}	Streptocycline @ 200 ppm	68.85 (43.51)	23.49 (13.59)
T_{20}	Control (Untreated)	90.00 (64.16)	-

	CD at 1%	1.02	1.57
	CV	1.37	5.17
	S.Em (±)	0.36	0.56

*Angular transformation, mean value of five replication

In vivo* evaluation of different chemicals on *Xanthomonas oryzae* pv. *oryzae

The results of Table 2 clearly indicated that, The minimum mean disease incidence *i.e*. 39.64% was recorded in treatment Copper oxychloride @ 0.3% + Streptocycline @ 0.005% followed by Copper hydroxide @ 0.3% + Streptocycline @ 0.005% (40.91%) while the maximum mean disease incidence was recorded in untreated control *i.e*. 72.73%. The maximum mean disease decrease over control *i.e*. 44.76% was found in treatment Copper oxychloride @ 0.3% + Streptocycline @ 0.005% followed by Copper hydroxide @ 0.3% + Streptocycline @ 0.005% *i.e*. 43.02%, At 50 days after transplanting (50 DAT), among all the treatments, Copper oxychloride @ 0.3% + Streptocycline @ 0.005% was significantly the minimum disease incidence 51.50% was recorded and was at par with Copper hydroxide 0.3% + Streptocycline @ 0.005% *i.e.* 52.53%. The maximum disease incidence was recorded in untreated control *i.e*. 67.68%.

At 70 days after transplanting (70 DAT), Copper oxychloride @ 0.3% + Streptocycline @ 0.005% was recorded the maximum percentage disease decrease over control *i.e* 39.44 and at par with Copper hydroxide 0.3% + Streptocycline @ 0.005% *i.e*. 38.03. The minimum disease incidence decrease over control was recorded in (Tebuconazole 50% + Trifloxystrobin 25%) @ 0.02% *i.e*. 21.12%.

At 90 days after transplanting (90 DAT), the maximum disease incidence was recorded in untreated control (73.74) and results indicated that, all the treatments were significantly superior to control. Copper oxychloride @ 0.3% + Streptocycline @ 0.005% was recorded least disease incidence *i.e.* 35.35% and was at par with Copper hydroxide 0.3% + Streptocycline @ 0.005% *i.e*. 37.37%.

At 110 days after transplanting (110 DAT), among the all treatments, Copper oxychloride @ 0.3% + Streptocycline @ 0.005% was recorded the maximum percent disease decrease over control *i.e* 63.64 and followed by Copper hydroxide 0.3% + Streptocycline @ 0.005% *i.e*. 62.34. The minimum percentage disease decrease over control was recorded in (Tebuconazole 50% + Trifloxystrobin 25%) @ 0.02% *i.e*. 42.86% as compared to control.

In present study, after testing all chemicals, it showed that Copper oxychloride @ 0.3% + Streptocycline @ 0.005% are highly effective against this plant pathogenic bacteria. Kulkarni and Jahagirdar (2011) [7] were supported, studied and recorded that application of Streptomycin sulphate @ 0.05% and Copper oxychloride @ 0.30% was recorded 16.29% and 22.66% respectively disease incidence against *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight disease of rice. Seed treatment with Streptocycline 100 ppm with foliar application of Streptocycline in combination with Copper oxychloride (100 + 500 ppm) was the most effective in minimizing disease incidence against *Xanthomonas oryzae* pv. *oryzae* (Biswas *et al*. 2009) [2].

Chaudhary *et al*. (2012) [3] studied and found that minimum disease incidence of bacterial leaf blight, in case of bordeaux mixture alone (40.89 and 44.06%) and in combination with Oxytetracycline (38.67% and 42.67%) and Streptomycin (36.89% and 39.55%) as compared to control (56.00% and 63.45%) in two consecutive years. Streptocycline @ 100 ppm recorded 46.72% disease incidence against *Xanthomonas oryzae* pv. *oryzae.* (Singh *et al*. 2014) [10]. Singh *et al*. (2015) [11] reported that combination of Copper oxychloride @ 500 g/ha + Oxytetracycline @ 75 g/ha and combination of Copper oxychloride @ 500 g/ha + Streptomycin @ 15 g/ha was found effective with 55.10% and 55.90% disease intensity respectively against *Xoo* causing bacterial leaf blight of rice. Combination of Copper oxychloride @ 0.25% + Streptocycline @ 100 ppm and Carbendazim @ 0.1% + Streptocycline @ 100 ppm were found highly effective against bacterial leaf blight of cotton and recorded 15.04% and 18.33% disease incidence respectively (Jagtap *et al*. 2012) [5].

Table 2: Effect of different chemicals on Disease Incidence of Bacterial leaf blight disease of rice

S. No	Treatments	Per cent disease incidence (50 DAT)	Disease decrease over control (%)	Per cent disease incidence (70 DAT)	Disease decrease over control (%)	Per cent disease incidence (90 DAT)	Disease decrease over control (%)	Per cent disease incidence 110 DAT)	Disease decrease over control (%)	Mean Disease incidence (%)	Mean disease decrease over control (%)
T_1	(Tebuconazole 50% + Trifloxystrobin 25%) Nativo @ 0.0%	65.66 (41.04)*	2.98	56.57 (34.45)	21.12	51.50 (31.00)	30.16	44.44 (26.38)	42.86	54.54 (33.22)	24.28
T_2	(Tebuconazole 50% + Trifloxystrobin 25%) Nativo @ 0.02% + Streptocycline @ 0.005%	62.63 (38.78)	7.46	53.54 (32.37)	25.34	47.43 (28.31)	35.67	39.39 (23.20)	49.35	50.75 (30.66)	29.46
T_3	(Azoxystrobin 25% + Difenoconazole 12.5%) Amistar top @ 0.03%	64.65 (40.28)	4.47	54.55 (33.06)	23.94	49.45 (29.64)	32.94	41.41 (24.46)	46.76	52.52 (31.86)	27.03
T_4	(Azoxystrobin 25% + Difenoconazole 12.5%) Amistar top @ 0.03% + Streptocycline @ 0.005%	61.62 (38.04)	8.95	51.50 (31.00)	28.19	46.44 (27.67)	37.02	36.36 (21.32)	53.25	48.98 (29.58)	31.85
T_5	[Mancozeb (12%) + Carbendazim (63%)] Sixer @ 0.15% + Streptocycline @ 0.005%	54.55 (33.06)	19.40	46.44 (27.67)	35.24	40.40 (23.83)	45.21	31.31 (18.25)	59.74	43.18 (25.70)	39.90
T_6	Copper oxychloride @ 0.3% + Streptocycline @ 0.005%	51.50 (31.00)	23.90	43.43 (25.74)	39.44	35.35 (20.70)	52.06	28.28 (16.43)	63.64	39.64 (23.46)	44.76
T_7	Copper hydroxide @ 0.3% + Streptocycline @ 0.005%	52.53 (31.69)	22.38	44.44 (26.38)	38.03	37.37 (21.94)	49.32	29.29 (17.03)	62.34	40.91 (24.26)	43.02
T_8	Copper oxychloride @ 0.3% + Oxytetracycline @ 0.005%	55.56 (33.75)	17.90	47.43 (28.31)	33.86	42.42 (25.10)	42.47	34.34 (20.08)	55.84	44.94 (26.81)	37.52
T_9	Copper hydroxide @ 0.3% + Oxytetracycline @ 0.005%	58.59 (35.87)	13.43	49.45 (29.64)	31.05	44.44 (26.38)	39.73	38.38 (22.57)	50.65	47.72 (28.61)	33.72

T_{10}	Control (Untreated)	67.68 (42.59)	-	71.72 (45.82)	-	73.74 (47.51)	-	77.78 (51.06)	-	72.73 (46.75)	
	S.Em (±)	0.69		0.40		0.68		0.44			
	CD (5%)	2.07		1.20		2.05		1.33			
	CV (%)	2.37		1.51		2.75		1.57			

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