The Pharma Innovation Journal 2022; 11(10): 1039-1042

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(10): 1039-1042

www.thepharmajournal.com
Received: 06-07-2022
Accepted: 12-09-2022

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In Vitro Evaluation of Ready-mix Fungicides against *Alternaria alternata* and *Xanthomonas citri pv. malvacearum* Causing Foliar Diseases in Bt Cotton

NS Chavda and NM Gohel

Abstract
Among the eleven ready-mix fungicides evaluated at two different concentrations by poisoned food technique under *in vitro* condition against *A. alternata* revealed azoxystrobin 18.2% + difenoconazole 11.4% SC and tebuconazole 50% + trifloxystrobin 25% WG at 500 and 1000 ppm concentrations significantly inhibited the mycelial growth of the pathogen and proved to be most effective. The highest zone of inhibition was achieved with streptomycin sulphate 90% + tetracycline hydrochloride 10% SP at 100 and 200 ppm concentrations against *X. citri* pv. *malvacearum*.

Keywords: alternaria alternate, xanthomonas citri pv malvacearum, poisoned food technique, agar well diffusion, bt cotton

Introduction
India is known as an agricultural region and agriculture is the main source of income for the majority of the population. India is a significant cotton producer. Cotton, also known as "White Gold" or "Emperor Fibers," is regarded as one of the best cash crops in the world. It is a valuable agricultural product that provides a source of income for millions of farmers in both developed and developing countries, as well as a means of subsistence for approximately sixty million people. The cotton crop is plagued by a variety of diseases that can be divided into two categories: foliar and soil-borne diseases. Uppal *et al*. (1935) [16] recorded the first case of cotton leaf spot (*A. macrospora* zimm.) in India, which was a major factor in the poor production of cotton, leading to bacterial blight caused by *X. campestris* pv. *malvacearum* and the boll rot complex, which are major constraints. This pathogen affects nearly every stage of the harvest, resulting in significant losses in seed cotton production, seed index, oil percentage and ginning outturn (Meshram and Sheo Raj, 1988; Shelke *et al*., 2012) [6, 12]. This work is aimed to study; newer ready mix fungicides against *Bt* cotton pathogens.

Materials and Methods
The diseased samples (leaves) of *Bt* cotton showing typical symptoms of foliar diseases *i.e.* Alternaria leaf spot (ALS) and bacterial blight (BB) were collected from infected *Bt* cotton fields during *Kharif*, 2019 and brought to the lab for microscopic examination and tissue isolation of the causative agents for further research.
The pathogenicity was proved under glasshouse conditions by artificial inoculation of pathogens *i.e. Alternaria* sp., *Xanthomonas* sp. Seeds of *Bt* cotton were surface sterilized with 1 percent sodium hypochlorite and sown in earthen pots containing sterilized soil and allowed to grow for a month. The plants were exposed to 95 percent humidity prior to inoculation for 24 hrs. Thereafter, they were inoculated separately with a spore suspension (5.4 x10^6 spores/ml) of *Alternaria* sp. Spore suspension spray inoculation. The *Xanthomonas* sp. were harvested using syringe inoculation plants were artificially inoculated by scraping the plate surface with sterilised distilled water at the six true-leaf stages by injecting the leaves (10^8 cfu/ml) on the lower surfaces into six inoculation points using a syringe without needle and applying constant pressure against the leaf until an area of mesophyll tissue water-soaked (Bielsa *et al.,* 2012) [1].

Isolation and Identification of the Pathogens Causing Foliar Diseases
The pathogens were isolated from foliar plant parts of *Bt* cotton. Standard tissue isolation procedure was followed for isolation of the fungal pathogens *i.e. Alternaria* sp. (Tuite, 1969) [15]

and cultures obtained were purified by hyphal tip method (Rangaswami, 1972) [9]. The cultures obtained were kept on PDA slants for further study. For isolation of *Xanthomonas* sp., the diseased portion of leaves was cut into small pieces (1x1 cm), surface sterilized with 1 percent sodium hypochlorite solution and then washed with distilled water. Bacteria associated with cotton leaves were obtained by streaking loopful of bacterial suspension prepared from water-soaked leaf lesions on NA medium (Salaheddin *et al.* 2005) [11]. After 36 hrs. of incubation at 27 °C temperature, single colonies were obtained which were further purified on NA, maintained and stored at 4 °C temperature.

The identification of pathogens causing foliar diseases of *Bt* cotton grown on PDA (*Alternaria* sp.) and NA medium (*Xanthomonas* sp.) were examined visually as well as microscopically for cultural and morphological characters *viz*., mycelial growth, colour and conidial characters (*Alternaria* sp.). The bacterial colony (*Xanthomonas* sp.) was examined under a microscope and identified using morphological characteristics (shape, size, texture, colony colour and Gram reaction).

Evaluation of Ready-Mix Fungicides against *A. alternata* and *X. citri* pv. *malvacearum* causing foliar diseases

Eleven fungicides with two different concentrations under *in vitro* of different chemical groups were tested separately for their effectiveness against *A. alternata* using poisoned food technique (Grover and Moore, 1962) [3] and agar well diffusion method (Murray *et al*., 1995) [7] for *X. citri* pv. *malvacearum*.

Experimental details

a)	Location	:	Department of Plant Pathology, BACA, AAU, Anand
b)	Design	:	Completely Randomized Design
c)	Treatments	:	12
d)	Repetitions	:	3
e)	Methods	:	Poisoned food technique (*A. alternata*) and Agar well diffusion method (*X. citri* pv. *malvacearum*)

Poisoned Food Technique (*A. alternata*)

A conical flask was filled with the required amounts of each test fungicides containing 100 ml melted PDA medium so as to get the required concentration in parts per million (ppm). The flask containing the poisoned medium was well shaken to facilitate a uniform mixture of fungicides and 15 ml was poured in each sterilized Petri plate. On solidification of the medium, the plates were inoculated in the centre by placing a 5 mm diameter culture disc cut aseptically with the help of a cork borer from seven days old pure culture of *A. alternata*. Three repetitions were kept for each concentration of the respective fungicide. The inoculated plates were incubated at 28±1 °C. The growth of test fungus on non-poisoned PDA was served as a control.

Observations Recorded

Observations on the radial growth were recorded from 24 hrs. of the incubation at 28±1 °C till the complete growth of test pathogen in control plates. The percent growth inhibition over control was calculated by using the formula given by Vincent (1947) [17].

$$\text{Growth inhibition (\%)} = \frac{DC - DT}{DC} \times 100$$

Where, DC = Colony diameter in control (mm)
DT = Colony diameter in respective treatment (mm)

Agar Well Diffusion Method (*X. citri* pv. *malvacearum*)

Seventy-two hours old bacterial pathogen *X. citri* pv. *malvacearum* (10^6 cfu/ml) was maintained in nutrient broth. Molten nutrient agar was seeded with bacterial culture maintained in nutrient broth @ 1 ml/100 ml of nutrient agar. Nutrient agar was poured into the sterilized Petri plates and allowed to solidify. A well (5 mm in diameter) was made by punching the nutrient agar with a sterilized cork borer on the corner of the plate in four directions by leaving a distance of 1 cm from the periphery of the plates. Each well was poured with 50 µl of various fungicides at different concentrations. Three repetitions were kept for each concentration of the respective fungicide. The growth of test bacteria on non-poisoned NA was served as a control. The efficacy of the fungicides was assessed by measuring the area of inhibition zone (mm) after 48 hrs. of incubation at 28±1 °C.

Observations Recorded

Inhibition zone (mm)

Table 1: Treatments details

Tr. No.	Treatments	Concentrations (ppm)	
T_1	Carboxin 37.5% + thiram 37.5% DS	500	1000
T_2	Azoxystrobin 8.3% + mancozeb 66.7% WG	500	1000
T_3	Metiram 55% + pyraclostrobin 5% WG	500	1000
T_4	Tebuconazole 50% + trifloxystrobin 25% WG	500	1000
T_5	Azoxystrobin 18.2% + difenoconazole 11.4% SC	500	1000
T_6	Fluxapyroxad 167 g/l + pyraclostrobin 333 g/l SC	500	1000
T_7	Pyraclostrobin 133 g/l + epoxiconaxole 50 g/l SE	500	1000
T_8	Azoxystrobin 11% + tebuconazole 18.3% SC	500	1000
T_9	Azoxystrobin 7.1% + propiconazole 11.9% SC	500	1000
T_{10}	Mancozeb 40% + azoxystrobin 7% OS	500	1000
T_{11}	Streptomycin sulphate 90% + tetracycline hydrochloride 10% SP	100	200
T_{12}	Control (Test pathogen only)		

Results and Discussion

Assessment of ready-mix fungicides against *A. alternata* And *X. citri* pv. *malvacearum* causing foliar diseases

Alternaria alternate

Out of tested fungicides, azoxystrobin 18.2% + difenoconazole 11.4% SC and tebuconazole 50% + trifloxystrobin 25% WG were found significantly superior at both concentrations (500 and 1000 ppm) with mycelial growth inhibition of 96.55, 97.63 and 93.19, 97.52 percent, respectively followed by pyraclostrobin 133 g/l + epoxiconazole 50 g/l SE registered 96.71 percent mycelial growth inhibition at 1000 ppm concentration. The next best treatment was fluxapyroxad 167 g/l + pyraclostrobin 333 g/l SC and azoxystrobin 8.3% + mancozeb 66.7% at 1000 ppm concentration with 94.24 and 93.68 percent mycelial growth inhibition, respectively.

The results of the observations on mycelial growth and percent growth inhibition (PGI) after fifteen days of incubation. When compared to the control, all of the fungicides significantly reduced the growth of *A. alternata*.

Earlier researchers, such as Indira *et al.* (2019) [4], Bodhke *et al*. (2019) [2] and Rajeswari and Balasupramani (2020) [8], found a similar set of outcomes.

Rajeswari and Balasupramani (2020) [8] evaluated the effectiveness of various fungicides against *A. alternata in vitro* at three different concentrations. Tebuconazole 50% + trifloxystrobin 25% WG was shown the most effective in inhibiting mycelial growt

Xanthomonas citri* pv. *malvacearum

The effects of the above-mentioned ready-mix fungicides were evaluated against *X. citri* pv. *malvacearum.*

Streptomycin sulphate 90% + tetracycline hydrochloride 10% SP was the most effective at both concentrations in inhibiting *X. citri* pv. *malvacearum* among the ready-mix fungicides tested. At both concentrations, 100 and 200 ppm, the inhibition zone measured 19.91 and 21.44 mm, respectively. The next better treatment was carboxin 37.5% + thiram 37.5% DS at 1000 ppm concentration producing an inhibition zone of 12.21 mm against *X. citri* pv. *malvacearum*.

The essence of the ready-mix fungicides, namely streptomycin sulphate 90% + tetracycline hydrochloride 10% SP, was found the most potent and was chosen for field application against bacterial blight disease based on its effectiveness.

The current findings are in line with the findings of scientists such as Singh *et al.* (2007) [13], Sonpriya (2016) [14], Sajid (2016) [10], and Kharat (2020) [5]. Singh *et al.* (2007) [13] assessed twelve fungicides by disc plate method against *X. campestris* pv. *malvacearum*. Among them, thiram 75% WS, carbendazim 12% + mancozeb 63% WP was found effective against pathogens. Kharat (2020) [5], who tested *in vitro* effectiveness of chemicals and antibiotics against *X. axonopodis* pv. *malvacearum* causing angular leaf spot of cotton.

Table 2: Effect of different ready-mix fungicides on the growth of *Alternaria alternata in vitro*

Trt. No.	Treatments	Conc. (ppm)	Mycelial growth (mm)	Growth inhibition (%)	Conc. (ppm)	Mycelial growth (mm)	Growth inhibition (%)
T_1	Carboxin 37.5% + thiram 37.5% DS	500	34.95^{f}	61.17	1000	29.21^{d}	67.54
T_2	Azoxystrobin 8.3% + mancozeb 66.7% WG	500	23.31^{e}	74.10	1000	5.69^{ch}	93.68
T_3	Metiram 55% + pyraclostrobin 5% WG	500	20.44^{d}	77.29	1000	8.36^{c}	90.71
T_4	Tebuconazole 50% + trifloxystrobin 25% WG	500	6.13^{b}	93.19	1000	4.23^{ba}	97.52
T_5	Azoxystrobin 18.2% + difenoconazole 11.4% SC	500	3.10^{a}	96.55	1000	2.13^{a}	97.63
T_6	Fluxapyroxad 167 g/l +pyraclostrobin 333 g/l SC	500	45.23^{g}	49.74	1000	5.18^{b}	94.24
T_7	Pyraclostrobin 133 g/l + epoxiconazole 50 g/l SE	500	17.17^{c}	80.92	1000	2.96^{cba}	96.71
T_8	Azoxystrobin 11% + tebuconazole 18.3% SC	500	88.55^{ih}	1.61	1000	76.02^{e}	15.53
T_9	Azoxystrobin 7.1% + propiconazole 11.9% SC	500	90.00^{i}	0.00	1000	86.49^{g}	3.91
T_{10}	Mancozeb 40% + azoxystrobin 7% OS	500	85.81^{h}	4.66	1000	82.11^{f}	8.77
T_{11}	Streptomycin sulphate 90% + tetracycline hydrochloride 10% SP	100	88.88^{i}	1.24	200	87.42^{hg}	3.06
T_{12}	Control (No fungicide)	-	90.00^{i}	-	-	90.00^{h}	-
	S. Em. ±	-	0.86	-	-	0.85	-
	CD at 5%	-	2.51	-	-	2.49	-
	C.V. (%)	-	3.02	-	-	3.71	-

Note: Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5% level of significance

Table 3: Effect of ready-mix fungicides on the inhibition of *Xanthomonas citri* pv. *malvacearum in vitro*

Trt. No.	Treatments	Conc. (ppm)	Zone of inhibition (mm)	Conc. (ppm)	Zone of inhibition (mm)
T_1	Carboxin 37.5% + thiram 37.5% DS	500	0.00^{b}	1000	12.21^{b}
T_2	Azoxystrobin 8.3% + mancozeb 66.7% WG	500	0.00^{b}	1000	0.00^{c}
T_3	Metiram 55% + pyraclostrobin 5% WG	500	0.00^{b}	1000	0.00^{c}
T_4	Tebuconazole 50% + trifloxystrobin 25% WG	500	0.00^{b}	1000	0.00^{c}
T_5	Azoxystrobin 18.2% + difenoconazole 11.4% SC	500	0.00^{b}	1000	0.00^{c}
T_6	Fluxapyroxad 167 g/l + pyraclostrobin 333 g/l SC	500	0.00^{b}	1000	0.00^{c}
T_7	Pyraclostrobin 133 g/l + epoxiconazole 50 g/l SE	500	0.00^{b}	1000	0.00^{c}
T_8	Azoxystrobin 11% + tebuconazole 18.3% SC	500	0.00^{b}	1000	0.00^{c}
T_9	Azoxystrobin 7.1% + propiconazole 11.9% SC	500	0.00^{b}	1000	0.00^{c}
T_{10}	Mancozeb 40% + azoxystrobin 7% OS	500	0.00^{b}	1000	0.00^{c}
T_{11}	Streptomycin sulphate 90% + tetracycline hydrochloride 10%	100	19.91^{a}	200	21.44^{a}

	SP				
T_{12}	Control (No fungicide)	-	00.00[b]	-	00.00[c]
	S. Em. ±	-	0.02	-	0.06
	CD at 5%	-	0.07	-	0.20
	C.V. (%)	-	2.52	-	4.24

Note: Treatment means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5% level of significance

Conclusion

The highest mycelial growth inhibition of *A. alternata* was achieved with azoxystrobin 18.2% + difenoconazole 11.4% SC and tebuconazole 50% + trifloxystrobin 25% WG at 500 and 1000 ppm, while streptomycin sulphate 90% + tetracycline hydrochloride 10% SP at 100 and 200 ppm concentrations registered the highest inhibition zone against *X. citri* pv. *malvacearum.*

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