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In vitro evaluation of ready-mix fungicides against *Alternaria* and *Xanthomonas citri* pv. *malvacearum* causing foliar diseases

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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 *Xanthomonas. citri* pv. *malvacearum*.

Keywords: Agar well diffusion, *Alternaria alternata*, *Bt* cotton, poisoned food technique, *Xanthomonas citri pv malvacearum*

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) 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). This work is aimed to study; newer ready mix fungicides against *Bt* cotton pathogens.

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 per cent sodium hypochlorite and sown in earthen pots containing sterilized soil and allowed to grow for a month. The plants were exposed to 95 per cent humidity prior to inoculation for 24 hrs. There after, they were inoculated separately with a spore suspension (5.4 x106 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 (108 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).

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) and cultures obtained were purified by hyphal tip method (Rangaswami, 1972). 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 per cent 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). 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.* C*itri* 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) and agar well diffusion method (Murray *et al.*, 1995) 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*) andAgar 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 0C. 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 0C till the complete growth of test pathogen in control plates. The per cent growth inhibition over control was calculated by using the formula given by Vincent (1947).

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

Where, DC = Colony diameter in control (mm)

DT = Colony diameter in respective treatment (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)		

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

Trt. No.	Treatments	Conc. ppm) (mm)	Mycelial growth (%)	Growth inhibition	Conc. (ppm) (mm)	Mycelial growth (%)	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 (p=0.05)	-	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

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

Trt. No.	Treatments	Conc. (ppm) (mm)	Inhibition zone	Conc. (ppm) (mm)	Inhibition zone
T_1	Carboxin (37.5%) + thiram (37.5% DS)	500	0.00b	1000	12.21^b
T_2	Azoxystrobin (8.3%) + mancozeb (66.7% WG)	500	0.00b	1000	0.00^c
T_3	Metiram (55%) + pyraclostrobin (5% WG)	500	0.00b	1000	0.00^c
T_4	Tebuconazole (50%) + trifloxystrobin (25% WG)	500	0.00b	1000	0.00^c
T_5	Azoxystrobin (18.2%) + difenoconazole (11.4% SC)	500	0.00b	1000	0.00^c
T_6	Fluxapyroxad (167 g/l) + pyraclostrobin (333 g/l SC)	500	0.00b	1000	0.00$_c$
T_7	Pyraclostrobin (133 g/l) + epoxiconazole (50 g/l SE)	500	0.00b	1000	0.00$_c$
T_8	Azoxystrobin (11%) + tebuconazole (18.3% SC)	500	0.00b	1000	0.00$_c$
T_9	Azoxystrobin (7.1%) + propiconazole (11.9% SC)	500	0.00b	1000	0.00$_c$
T_{10}	Mancozeb (40%) + azoxystrobin (7% OS)	500	0.00b	1000	0.00$_c$
T^{11}	Streptomycin sulphate (90%) + tetracycline hydrochloride (10% SP)	100	19.91a	200	21.44$_a$
T^{12}	Control (No fungicide)	-	00.00b	-	00.00$_c$
	S. Em. ±	-	0.02	-	0.06
	CD (p=0.05)	-	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

Assessment of ready mix fungicides against *A. alternata* and *X. citri* pv. *malvacearum* Causing foliar diseases

Alternaria alternata

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 per cent, respectively followed by pyraclostrobin (133 g/l) + epoxiconazole (50 g/l SE) registered 96.71 per cent 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 per cent mycelial growth inhibition, respectively.

The results of the observations on mycelial growth and per cent 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), Bodhke *et al.* (2019) and Rajeswari and Balasupramani (2020), found a similar set of outcomes.

Rajeswari and Balasupramani (2020) 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), Sonpriya (2016), Sajid (2016), and Kharat (2020). Singh *et al.* (2007) 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), who tested in vitro effectiveness of chemicals and antibiotics against *X. axonopodis* pv. *malvacearum* causing angular leaf spot of cotton.

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 ppmconcentrations registered the highest inhibition zone against *X. citri* pv. *malvacearum.*

REFERENCES

Bielsa PA, Pothier JF, Roselló M, Duffy B, López MM. 2012. Detection and identification methods and new tests as developed and used in the framework of cost 873 for bacteria pathogenic to stone fruits and nuts *Xanthomonas arboricola* pv. *pruni. J. Plant Pathology.* **135** :146.

Bodhke VS, Patil, CU, Zade SB. 2019. Evaluation of fungicides and bioagents against *Alternaria macrospora* incitant of Alternaria blight of cotton. *Int. J. Chem. Stu.* **7** :237.

Grover RK, Moore JD. 1962. Toxicometric studies of fungicides against brown rot organism, *Sclerotinia fruiticola* and *S. laxa. Phytopathology.* **52**:876.

Indira SA. Sreedevi SC, Yenjerappa ST, & Shivaleela B. 2019. In vitro evaluation of different fungicides against *Alternaria macrospora* causing leaf spot of cotton. *Int. J. Chem. Stu.* **7**: 444-47.

Kharat KY. 2020. Management of bacterial blight (*Xanthomonas axonopodis* pv. *malvacearum*) in rainfed *Bt* cotton. Doctoral thesis. Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra. **87**.

Meshram MK, Sheo Raj. 1988. Assessing losses due to bacterial blight diseases. Plant Pathology, *The Tata McGraw Hill Publ. Co. Ltd., New Delhi.* 315.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR. 1995. Manual of Clinical Microbiology (6th ed.). Washington DC: *ASM Press.* 15-18.

Rajeswari E, Balasupramani P. 2020. In vitro evaluation of plant extracts, biocontrol agents and fungicides against leaf blight in pigeonpea. *J. Pharm. Phytoch.* **9**:84-88.

Rangaswami G. 1972. Diseases of crop plants in India. *Prentice-Hall of India Pvt. Ltd.*, New Delhi, India. 408.

Sajid M. 2016. Biochemical and physiological factors conducive for the development of bacterial blight of cotton and its management. *Doctoral Thesis*. University of Agriculture, Faisalabad, Pakistan. 62.

Salaheddin K. Marimuthu T, Ladhalakshmi D, Rabindran, R, Velazhahan R. 2005. A simple inoculation technique for evaluation of cotton genotypes for resistance to bacterial blight caused by *Xanthomonas axonopodis* pv. *malvacearum. J. Plant Disease and Prot.* **112**: 321-28.

Shelke GV, Aurangabadkar LP, Kashikar AR, Wadyalkar SR, Phalak MS, Khsrkar HH, Umslkar GV. 2012. Identification of resistance source for bacterial blight disease caused by *Xanthomonas axonopodis* pv. *malvacearum* and its genetic inheritance in upland cotton. *Cotton Research Journal*. **3** :167-73.

Singh A, Srivastava SSL, Akram M. 2007. Studies on bacterial leaf blight of cotton (*Gossypium* sp.). *Int. J. Sust. Crop Prod.* **2** :25-29.

Sonpriya PS. (2016). Studies on bacterial blight of cotton. *M.Sc. Thesis*. Vasantrao Naik Marathwada Agricultural University, Parbhani. 115.

Tuite JC. 1969. Plant pathological methods: fungi and bacteria. *Burgess publishing company*, Minneapolis, USA. 501.

Uppal BN, Patel MK, Kamat MM. 1935. The fungi of Bombay. Department of Agriculture, Bulletin. 176 :28.

Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1**59** :850.

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