Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com
ISSN Online: 2374-8362

Research Article | Journal of Advanced Research in Biotechnology | Open Access

Bactericidal potential of Agrochemicals against bacterial leaf spot pathogen *Xanthomonas campestris* pv. *Vesicatoria* of tomato prevalent in Nashik region, Maharashtra and ability of bacteria to form pesticide resistant mutant

Ajayasree T S*, S G Borkar And B G Barhate

Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Agriculture University, Rahuri, 413 722, Dist- Ahmednagar, Maharashtra state

Received:August 27, 2018; Accepted: November 14, 2018; Published: November 15, 2018

***Corresponding author:** AJAYASREE T S, Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Agriculture University, Rahuri, 413 722, Dist- Ahmednagar, Maharashtra state; E-mail id: ajayasree128@gmail.com*

Abstract

Selection of suitable agrochemicals/pesticides plays an important role in the management of bacterial plant pathogen prevalent in a given area, may be due to the presence of pesticide resistance available in the pathogen. In this scenario, the assessment of bactericidal potential of agrochemical to be used in the management of bacterial plant pathogen is very necessary. To manage the bacterial leaf spot and blight pathogen *Xanthomonas campestri*s pv. Vesicatoria on tomato prevalent in Nashik region, the bactericidal potential of various agrochemicals were assayed.

The bacterial potential of antibiotic Streptomycin sulphate, and Streptocycline; the bactericide Bactericin-100, and the Bordeaux mixture; the copper fungicide Copper-ox chloride and the di-thiocarbamate group of fungicide Mancozeb; the combination of Copper ox chloride + Streptocycline were against the tomato leaf spot and blight pathogen *Xanthomonas campestris* pv. *Vesicatoria* under *in vitro* condition. The minimum inhibitory concentration of these agrochemicals against *Xanthomonas campestris* pv. *Vesicatoria* varies indicating the variable bactericidal potential of these agrochemicals against the said bacterium prevalent in Nashik areas.

The bacterial population of *Xanthomonas campestris* pv. *Vesicatoria* was resistant to the antibiotic Terramycin, Streptomycin sulphate and to the fungicide Copper ox chloride, Mancozeb and a combination of Copper ox chloride + Streptomycin sulphate and induced the pesticide resistant mutant in the bacterium with various mutation frequencies. The mutation rate for Terramycin and Streptomycin sulphate was 3.5 x 10^{-4} and 2 x 10^{-4} respectively, whereas, for Dithane Z-78, Mancozeb and a combination of Copper ox chloride+ Streptomycin sulphate was 2.6 x 10^{-4}, 8 x 10^{-5} and 5 x 10^{-5}.respectively. The agrochemicals which were completely inhibitory to the bacterium and did not allow the formation of antibiotic or antibiotic + fungicide resistance mutant were Streptocycline, Streptocycline+ Copper ox chloride and Streptocycline + Bordeaux mixture.

Keywords: Antibiotics; Bactericides; Fungicides; Mutant

Introduction

Bacterial plant pathogens cause considerable losses due to their infection and spread under favorable climatic conditions (Borkar and Yumlembam 2016). The success of management of bacterial plant pathogens depends on the selection of suitable and effective agrochemicals/pesticides to control them. Most of the times, the bacterial plant pathogen develops the pesticide resistance to certain pesticides under field conditions. The development of this pesticide resistance can be a location specified in the crop region/areas depending upon the bacterial strains of the pathogen. Therefore, it is very necessary to know the efficacy of agrochemicals/pesticides against the bacterial pathogen in the given location.

Bacterial leaf spot and blight is a serious disease of tomato crop in tomato growing areas of Nashik district in Maharashtra state, India. The bacterial pathogen *Xanthomonas campestris* pv. *Vesicatoria* is prevalent in this region for over two decades (Borkar and Yumlembam 2016) in spite of much application of the pesticides on the crop to manage this bacterial disease. This may be due to the development of pesticide resistance in the bacterium, thereby making the pesticide/agrochemicals ineffective in its management. The rate of formation of pesticide-resistant mutant against the pesticide also varies with the pesticide and the strain of the pathogen (Anderson 2006; Araujo et al. 2012). Therefore, in the present investigation, the bactericidal potential of agrochemicals used by the farmers against the bacterial leaf spot pathogen *Xanthomonas campestris* pv. *vesicatoria* of tomato prevalent in Nashik region, Maharashtra and the ability of the bacterial population to form pesticide resistant mutant against these agrochemicals was studied.

 ***Corresponding author email:** ajayasree128@gmail.com*

Material and Methods

Isolation of bacterial pathogen from infected tomato leaves

The isolation of bacteria responsible for leaf spot and blight in tomato leaves, collected from the infected tomato crop in Nashik district of Maharashtra, was done on nutrient agar media by routine bacteriological laboratory techniques of isolation of plant pathogenic bacteria (Borkar 2018). The bacterium was identified as *Xanthomonas campestris* PV. *Vesicatoria* based on the identification test (Borkar 2018).

The purified bacterial culture was assayed under *in vitro* condition for its sensitivity to different agrochemicals used by the farmers in the tomato field. The routine poison food technique was used to determine the bactericidal potential of these agrochemicals against the bacterium *Xanthomonas campestris* PV. *Vesicatoria*

The bactericidal potential of different agrochemicals against tomato bacterium *Xanthomonas campestris* PV. *Vesicatoria*

The bactericidal potential of antibiotics

The antibiotics viz. Streptomycin sulphate, Streptocycline, and Terramycin were evaluated for its efficacy against the tomato bacterium at a concentration of 25, 50, 75, 100, 250, 500 and 1000 ppm.

The bactericidal potential of bactericides

Two bactericides viz. Bactericin-100 and Bordeaux mixture was evaluated for its efficacy against the bacterium. Bactericin-100 was evaluated at a concentration of 25, 50, 75, 100, 250 and 500 ppm; whereas the Bordeaux mixture was evaluated at 0.01, 0.025, 0.05, 0.1 and 0.2% concentration.

The bactericidal potential of fungicides

Three fungicides viz. Copper ox chloride, Mancozeb, and Zineb were evaluated for its efficacy against the bacterium as these fungicides are commonly used in the tomato field by the farmer for control of different fungal diseases including bacterial leaf spot.

The fungicides Copper ox chloride, Mancozeb, and Zineb were evaluated at 0.01, 0.025, 0.05, 0.1 and 0.2 per cent concentration.

Bactericidal potential of combination of fungicides + antibiotics

Three combinations particularly copper ox chloride + Streptocycline, Bordeaux mixture + Streptocycline and Copper ox chloride + Streptomycin sulphate were evaluated for their efficacy against the bacterium by poison food technique. A combination of Copper ox chloride + Streptocycline was evaluated at 0.025 % + 50 ppm, 0.05 % + 50 ppm and 0.2 % + 50 ppm. A combination of Bordeaux mixture + Streptocycline was evaluated at 0.025 % + 50 ppm and 0.05 % + 50 ppm and a combination of Copper ox chloride + Streptomycin sulphate was evaluated at 0.025 % + 50 ppm, 0.05 % + 75 ppm and 0.2 % + 500 ppm respectively.

Studies on pesticide resistant mutant inducing ability in the bacterium

A bacterial suspension of Xanthomonas campestris PV. Vesicatoria was prepared from the 24 hrs old bacterial growth in sterile distilled water and the optical density of bacterial suspension was adjusted to 0.1 which content 107cfu/ml (Borkar 2018). 1 ml of this suspension was mixed in the 25 ml poison nutrient medium of above agrochemicals of minimum inhibitory concentration for the bacteria and poured in the sterile Petri plate. The medium in the plates was solidified in the slanting position and the plates were incubated at 280C in BOD incubator. The formation of pesticide-resistant mutant colonies was observed after a week in the plates and the mutant population was calculated to determine the rate of mutation.

Result and Discussion

The bactericidal potential of different agrochemicals against tomato bacterium *Xanthomonas campestris* pv. *Vesicatoria*

Table 1: The bactericidal potential of antibiotics on tomato leaf spot pathogen *Xanthomonas campestris* pv. *Vesicatoria*

Sr.No	Antibiotics	Concentration (ppm)	Bactericidal potential	MIC
1.	Streptomycin sulphate	1000	–	1000 ppm
		500	+	
		250	+	
		100	+	
		75	+	
		50	+	
		25	+	
2.	Streptocycline	500	–	25 ppm
		250	–	
		100	–	
		75	–	
		50	–	
		25	–	
3.	Terramycin	500	–	75 ppm
		250	–	
		100	–	
		75	–	
		50	+	
		25	+	

+ = Bacterial growth; – = inhibition of bacterial growth

The bactericidal potential of antibiotics

The antibiotic Streptocycline was effective at a very low concentration of 25 ppm whereas the antibiotic Terramycin was effective at a concentration of 75 ppm and Streptomycin sulphate at a concentration of 1000 ppm to inhibit the bacterial growth of *Xanthomonas campestris* pv. *Vesicatoria*

The minimum inhibitory concentration (MIC) for Streptomycin sulphate was 1000 ppm, for Streptocycline 25 ppm and for Terramycin 75 ppm. Below the MIC, there was a bacterial growth in the poison food plates (Table 1).

The bactericidal potential of bactericides

The bactericide Bactericin-100 was effective at a concentration of 50ppm and above while it was not effective at 25 ppm. The Bordeaux mixture was effective at a concentration of 0.1 percent but it was not effective at 0.05 % to inhibit the growth of the bacterium. The MIC for Bactericin-100 was 50 ppm whereas for Bordeaux mixture it was 0.1 percent (Table 2).

Table 2: The bactericidal potential of bactericides on tomato leaf spot pathogen *Xanthomonas campestris* pv. *Vesicatoria*

Sr.No	Bactericides	Concentration	Bactericidal potential	MIC
1.	Bacteriocin -100	500	–	50 ppm
		250	–	
		100	–	
		75	–	
		50	–	
		25	+	
2.	Bordeaux mixture	0.2	–	0.1 %
		0.1	–	
		0.05	+	
		0.025	+	
		0.01	+	

+ = **Bacterial growth**; – = **inhibition of bacterial growth**

The bactericidal potential of fungicides

The fungicide Copper ox chloride was effective at 0.1 percent and above concentration. The fungicide Mancozeb was effective at 0.05 percent and Zineb was effective at 0.025 percent concentration to inhibit the bacterial growth (Table 3).

The bactericidal potential of a combination of fungicides + antibiotics

A combination of Copper ox chloride + Streptocycline was effective at 0.025 % + 50 ppm indicating that the concentration of Copper ox chloride can be lowered down when used with Streptocycline. A combination of Bordeaux mixture + Streptocycline was effective at 0.025 % + 50 ppm indicating that the concentration of the Bordeaux mixture can also be lowered down when mixed with Streptocycline. The combination of Copper ox chloride + Streptomycin sulphate was effective at

Table 3: The bactericidal potential of copper and di-thiocarbamate group fungicides on tomato leaf spot pathogen *Xanthomonas campestris* pv. *Vesicatoria*

Sr.No	Fungicide	Concentration (%)	Bactericidal potential	MIC
1.	Copper ox chloride	0.2	–	0.1%
		0.1	–	
		0.05	+	
		0.025	+	
		0.01	+	
2.	Mancozeb	0.2	–	0.05%
		0.1	–	
		0.05	–	
		0.025	+	
		0.01	+	
3.	Zineb	0.2	–	0.025%
		0.1	–	
		0.05	–	
		0.025	–	
		0.01	+	

+ = **Bacterial growth**; – = **inhibition of bacterial growth**

Table 4: The bactericidal potential of fungicide + antibiotics on tomato leaf spot pathogen *Xanthomonas campestris* pv. *Vesicatoria*

Sr.No	Combination	Concentration	Bactericidal potential	MIC
1.	Copper ox chloride + Streptocycline	0.2 % + 50 ppm	–	0.025 % + 50 ppm
		0.05 % + 50 ppm	–	
		0.025 % + 50 ppm	–	
2.	Bordeaux mixture + Streptocycline	0.05 % + 50 ppm	–	0.025 % + 50 ppm
		0.025 % + 50 ppm	–	
3.	Copper ox chloride + Streptomycin sulphate	0.2 % + 500 ppm	–	0.2 % + 500 ppm
		0.05 % + 75 ppm	+	
		0.25% + 50 ppm	+	

+ = **Bacterial growth**; – = **inhibition of bacterial growth**

0.2 % + 500 ppm and not below this concentration. The MIC for Copper ox chloride with Streptocycline combination was 0.025 % + 50 ppm, for Bordeaux mixture with Streptocycline combination it was 0.025 % + 50 ppm and for Copper ox chloride, with Streptomycin sulphate combination it was 0.2 % + 500 ppm (Table 4).

Pesticide resistant mutant inducing ability in the population of *Xanthomonas campestris* PV. *Vesicatoria*

The bacterial population of *Xanthomonas campestris* PV. *Vesicatoria* was resistant to the antibiotic Terramycin, streptomycin sulphate and to the fungicide Copper ox chloride, Mancozeb and a combination of Copper ox chloride + Streptomycin sulphate and induced the pesticide resistant mutant in the bacterium. The mutation rate for Terramycin and Streptomycin sulphate was 3.5 x 10^{-4} and 2 x 10^{-4} respectively, whereas, for Dithane Z-78, Mancozeb and a combination of Copper ox chloride+ Streptomycin sulphate was 2.6 x $10^{-4,}$ 8 x 10^{-5} and 5 x 10^{-5}.respectively. The agrochemicals which were completely inhibitory to the bacterium and did not allow the formation of antibiotic or antibiotic + fungicide resistance mutant were Streptocycline, Streptocycline+ Copper ox chloride and Streptocycline + Bordeaux mixture.

Several workers had studied the potential of agrochemicals as bactericide (Patyka et al. 2012; Hulloli et al. 1998; Verma et al. 1992; Nafde and Verma 1984; Verma and Singh 1976) and formation of pesticide resistance in bacterial plant pathogens (Marques et al. 2009; Cazrola et al. 2002; Ritchie and Dittapongpitch 1991; Adaskaveg and Hine 1985).

Jones et al. (1991) monitored the populations of copper-resistant (Cur) strains of *Xanthomonas campestris* PV. *Vesicatoria* in the field on non-symptomatic tomato leaflets treated with copper or with a copper and Mancozeb mixture over three and four seasons, respectively. In a greenhouse study, where a Cur strain of *Xanthomonas campestris* PV. *Vesicatoria* was applied to tomato foliage; bacterial populations were significantly less on plants treated with copper or with a copper and Mancozeb mixture than on untreated plants. However, leaflets treated with the copper and Mancozeb combination had significantly lower Cu^r populations than leaflets treated with copper alone. Bouzar et al. (1999) found *Xanthomonas* isolates resistant to both Streptomycin and copper in the Caribbean and Central America. Buonaurio et al. (1994) reported few strains of race1, race 2 and race 3 of *Xanthomonas campestris* PV. *Vesicatoria* tolerant to Copper sulphate (200 µg/ml), while susceptible to Streptomycin sulphate (10 µg/ml)

Chand et al. (1994) reported widespread resistance in isolates of Xanthomonas campestris PV. Vesicatoria to copper and zinc collected in India during 1991-92. Stall and Thayer (1962) stated that resistance to streptomycin in Xanthomonas was found a century ago in Florida (USA).

Schroth et al. (1979) reported the increased concentrations of Streptomycin in media up to 1,000µg/ml increased the generation times of Streptomycin resistant strains but did not prevent growth. Virulence among Streptomycin resistant and Streptomycin susceptible strains varied but there was no consistent difference between the two groups. The Streptomycin resistant strains appeared to be relatively stable and were detected in orchards 6 years after termination of Streptomycin application.

Marco and Stall (1983) also reported the copper resistance in bacterial leaf spot diseases in Florida. Adaskaveg and Hine (1985) isolated the copper-sensitive strains of *Xanthomonas campestris* PV. *Vesicatoria* from infected pepper plants from two locations in Arizona where there was limited use of copper bactericides. Three copper tolerant strains of the bacterium were also isolated from diseased plants from the West Coast and Central Mexico, where copper bactericides have been used for more than 30 years. The Arizona strains were sensitive to various copper formulations (Copper hydroxide, Copper sulphate, Copper ammonium carbonate, and Basic copper sulphate) with and without the addition of Mancozeb as determined by the presence of inhibition zone in disk assays.

Bender et al. (1990) evaluated the efficacy of copper bactericides for control of *Xanthomonas campestris* PV. *Vesicatoria* in eastern Oklahoma tomato fields. Copper bactericides did not provide adequate control, and copper resistant strains of the pathogen were isolated. Ritchie (2000) reported that chemical control of *Xanthomonas vesicatoria* is limited to copper or copper combined with Maneb sprays that provide only marginal success due to the formation of resistant mutant thus making the disease very difficult to control once the epidemic is underway.

References

1. Adaskaveg JE and Hine RB. Copper tolerance and zinc sensitivity of Mexican strains of Xanthomonas campestris PV. vesicatoria, the causal agent of bacterial spot of pepper. Plant Dis. 1985;69(11):993-996.
2. Anderson DI. The biological cost of mutational antibiotic resistance: any practical conclusion. Curr Opin Microbiol. 2006;9(5):461-465.
3. Araújo ER, Costa JR, Ferreira MA and Quezado-Duval AM. Simultaneous detection and identification of the Xanthomonas species complex associated with tomato bacterial spot using species-specific primers and multiplex PCR. J Appl Microbiol. 2012;113(6):1479-1490. doi: 10.1111/j.1365-2672.2012.05431.x
4. Bender L, Malvick K, Conway E, George S and Pratt P. Characterization of pXV10A, a copper resistance plasmid in Xanthomonas campestris pv. Vesicatoria. Appl Environ Microbiol. 1990;56(1):170-175.
5. Borkar SG. Laboratory technique in plant bacteriology. CRC Press, U S A. 2018;320.
6. Borkar SG and Yumlembam RA. Bacterial Diseases of Crop Plants. CRC Press, U S A. 2016;594.

7. Bouzar H, Jones JB, Stall RE, Louws FJ, Schneider M, Rademaker JL, Bruijn FJ, et al. Multiphasic analysis of Xanthomonads causing bacterial spot disease on tomato and pepper in the Caribbean and Central America: evidence for common lineages within and between countries. Phytopathology. 1999;89(4):328-335. doi: 10.1094/PHYTO.1999.89.4.328
8. Buonarurio R, Stravato VM and Scortichni M. Characterization of Xanthomonas campestris pv. Vesicatoria from Capsicum annum L. in Italy. Plant Dis. 1994;78(3):296-299.
9. Cazrola FM, Arrebola E, Sesma A, Perez-Garcia A, Codima JC, Murillo J and de Vicente A, et al. Copper resistant in Pseudomonas syringe strain isolated from mango is encoded mainly in plasmids. Phytopathology. 2002;92(8):909-916. doi: 10.1094/PHYTO.2002.92.8.909
10. Chand R, Singh R and Singh PK. Distribution of pathogenic groups and races in Xanthomonas campestris pv. vesicatoria in peninsular India. Indian Phytopath.1994;47(3):251-252.
11. Hulloli SS, Singh RP and Verma JP. Management of bacterial blight of cotton with the use of neem based formulations. Indian Phytopath. 1998;51(1):21-25.
12. Jones JB, Woltz SS, Jones JP and Portier KL. Population dynamics of Xanthomonas campestris pv. vesicatoria on tomato leaflets treated with copper bactericides. Phytopathology. 1991;81(7):714-719.
13. Marco GM and Stall RE. Control of bacterial spot of pepper initiated by strains of Xanthomonas campestris pv. Vesicatoria that differ in sensitivity to copper. Plant Dis. 1983;67(7):779-781.
14. Marques E, Uesugi CH, and Ferreira MASV. Sensitivity to copper in Xanthomonas campestris pv. vesicatoria. Trop Plant Pathol. 2009;34(6):406-411.
15. Nafde SD and Verma JP. Effect of chemicals on translocation of streptomycin in the cotton seedling. Indian Phytopath. 1984;37:524-528.
16. Patyka V, Buletsa N, Pasichnyk L, Zhitkevish N, Kalinichenko A, Gnatiuk T and Butsenko L, et al. Specifics of pesticide effects on the phytopathogenic bacteria. Ecol Chem Eng S. 2012;23(2):311-331.
17. Ritchie DF and Dittapongpitch V. Copper and streptomycin resistant strains and host differentiated races of Xanthomonas campestris pv. vesicatoria. Plant Dis.1991;75:733-736.
18. Ritchie DF. Bacterial spot of pepper and tomato. The Plant Health Instructor.2000; doi: 10.1094/PHI-I-2000-1027-01
19. Schroth MN, Thomson SV and Moller WJ. Streptomycin resistance in Erwinia amylovora. Phytopathology.1979;69(1):565-568.
20. Stall RE and Thayer PL. Streptomycin resistance of the bacterial spot pathogen and control with streptomycin. Pl Dis Rep. 1962;46(1):389-392.
21. Verma J P and Singh RP. Chemical control of bacterial diseases of plants. Chemical Concept.1976;4:31-50.
22. Verma JP, Singh RP, Jindal JK and Trivedi BM. Bacterial plant pathogens and their management. Fusion Asia.1992;8:29-36.