



## Original Article

# Non-Hazardous Management of *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye in Chilli (*Capsicum* spp.) Using Leaf extracts of Medicinal Plants

D K Sharma \*

Vardhaman Mahaveer Open University, Kota, Rajasthan, India

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The leaf extracts of several medicinal plants were used to study their antimicrobial strength against *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye in chilli. Plant extracts at various concentrations (100, 50, 30 or 25%) were used to evaluate their antibacterial effects using filter paper disc assay, seeded agar method but in case of seed treatment *Tabernaemontana divaricata* and seed treatment methods. Aqueous leaf extracts of *Parthenium hysterophorus* and *Lantana camara* individually were found most effective against the pathogen in filter paper and seeded agar method improve seed germination and control of pathogen at 100% concentration as compared to check. The present study indicates the bio-efficacy of botanicals in effectively controlled of the pathogen or reduction the disease incidence significantly.

**Keywords:** *in vitro* study, antibacterial activity, filter paper disc assay, seeded agar method, standard blotter method, bacterial incidence, aqueous leaf extracts, *Xanthomonas axonopodis* pv. *vesicatoria*.

## 1. INTRODUCTION

Bacterial leaf spot (BLS) disease of sweet pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) is a major disease in tropical and subtropical climates appeared on mature and green fruits caused by *Xanthomonas axonopodis* pv. *vesicatoria*. It is reported from several countries of eastern and southern Africa, USA, Ethiopia, Kenya, Malawi, Mozambique and South Africa<sup>1,2</sup>. XAV suspected from symptoms on tomato and sweet pepper fruit was confirmed by isolation on semi-selective media including Tween B<sup>3</sup>. In

Corresponding author \*

Dr D K Sharma

Vardhaman Mahaveer Open University, Kota, Rajasthan, India

E-Mail: drdilipsharma12@gmail.com

India, bacterial leaf spot disease was first reported from Pune, Maharashtra in 1948 by Patel *et al.* in chilli. In Rajasthan, the disease caused 7.5 to 16.6 per cent loss in the yield of fruits and pathogen required a temperature range of 22-34°C with high humidity for maximum infection<sup>4,5</sup>.

*Xanthomonas axonopodis* pv. *vesicatora* (Doidge) Dye (syn: *Xanthomonas campestris* pv. *vesicatoria*) (XAV) is a gram-negative, rod-shaped bacterium that produce spot on leaf, fruit and cankers on stem. The disease occurs universally are relatively warm and moist environment cause lesions which reduce quality and nutritive value of fresh fruit for sale and processing<sup>6-10</sup>.

The disease started early in the crop before flowering and losses in marketable fruits may be more than 50%<sup>8,11</sup>. The pathogen was found seed-borne (10-15%) also subsists on infected plant debris, weeds and volunteer tomato plants<sup>12</sup>. The incidence was less than 5% persisted from one season to next in crop debris or on weed hosts<sup>13</sup>. The pathogen has been reported to be seed-borne in chilli.

## 2. MATERIALS AND METHODS

### Collection of plant materials

Plant leaves of selected seven plants were collected from Jaipur, Rajasthan, India for this experiment (Table 1, 2). The seven medicinal plants leaf extracts namely mexican prickly poppy (peeli kataili, *Argemone mexicana* L.) of family Papaveraceae, crape jasmine (chandani, *Tabernaemontana divaricata*) of family Apocynaceae, glorybower (bharangi, *Clerodendrum inerme*) of Verbenaceae, Madagascar periwinkle (sadabahar, *Catharanthus roseus*) of Apocynaceae, Lantana (Kuri, *Lantana camara*) of Verbenaceae, withania (ashwagandha, *Withania somnifera*) of Solanaceae, congress grass (gajar ghas, *Parthenium hysterophorus*) of Asteraceae were tested *in vitro* for control of pathogen. For the experiments 10 g fresh leaves of each plant was taken, washed thoroughly by distilled water followed with tap water. Now the surface sterilized leaves were crushed in sterile distilled water at the rate of 1g tissues in 1 ml of water (1: 1 w/v) using pestle and mortar. The extract was filtered through double layered cheesecloth and this filtrate was treated as stock solution.

### Seed treatment in SBM

Two seed samples of chilli, Ca-1227 and Ca-1234 naturally infected with XAV were treated individually with the aqueous leaf extracts of 7 medicinal plants for 4 hrs in two different concentrations, pure (100%, w/v) and diluted (30% v/v) in triplicate (100 seeds/ sample) were used. Seeds soaked in sterile distilled water were treated as check. All the treated and untreated seeds were incubated on moistened blotter papers and per cent seed germination, seedling symptom, incidence of the bacteria and inhibition of the pathogen observed on 8<sup>th</sup> days in standard blotter method<sup>17</sup>. The percent control of pathogens was calculated by the following formula-

[Percent control = Incidence in check (C) - incidence in treatment (T) /Incidence in check (C) x 100]

### Filter paper disc assay and seeded agar method

Two another methods namely seeded agar method and filter paper disc method (disc diffusion method) were also carried out to find out the antibacterial activity against XAV<sup>18-20</sup>. Bacterial suspension (10 ml) of test bacterium was spread by a sterile L-rods or cotton swab on nutrient agar medium. Filter paper discs of 8 mm diameter impregnated with plant extracts were placed in the inoculated plates. Filter paper discs soaked in double sterile distilled water placed in the middle of the plate used as check. The plates were incubated at 30±2° C for 48 hrs. In seeded agar method the wells (8 mm diameter) on nutrient agar medium (already seeded) using sterilized cork borer were yielded. The (1 ml v/v) crude form of suspension of leaf extracts was place in wells by using sterile syringe. The diameter or zone of inhibition was recorded upto 6 days in intervals of 24 hrs at 25 ± 2°C for each test agent.

The inhibition zones were measured in diameter (mm) around of the discs and calculated to compare the antibacterial activity of the test plant extracts. The inhibition annulus was calculated by following formula<sup>19-21</sup>-

[Activity index (AI) = Inhibition zone of sample/ Inhibition zone of standard]

The bacterium was identified by available detailed description on the basis of morphological and biochemical characteristics<sup>14, 15, 22-26</sup>.

## 3. RESULTS AND DISCUSSION

Plants and their derivatives are good alternative source of agrochemicals in managing bacterial diseases using secondary metabolites or bioactive compounds as alkaloids, flavonoids, tannins, saponins and terpenoids and can be used as source of natural pesticides<sup>27, 28</sup>. Medicinal plant extracts are eco-friendly and economical sources to control the plant diseases. Currently the scientific research showed much interest in control of various microbes or pests by exploit the antibacterial activity of medicinal plants to reduce the toxicity of chemicals to manage the plant diseases. The chemicals having several hazardous tend to accumulate in animal tissues posing threat to human health. Green plants are effective chemotherapeutants and can provide valuable sources of natural pesticides<sup>29, 30</sup>. Plant extract have a potential as environmentally safe alternatives chemicals in integrated disease management programs<sup>31</sup>. The bioactive and biochemical compounds of medicinal plant are of origin of non-phytotoxic and easily biodegradable<sup>35-39</sup>. Leaf extracts of various plants are known to possess antimicrobial activity<sup>32-34</sup>.

In the present study, aqueous leaf extracts of 7 medicinal plants tried and found effective to control the bacterial pathogen XAV to control by *Parthenium hysterophorus* followed by *Lantana camara* in filter paper method and seeded agar method gave best results at 100% concentration.

In standard blotter method *Tabernaemontana divericata* and *P. hysterothorus* found most effective at 100% concentration as compared to check. The maximum improvement in seed germination was shown by *P. hysterothorus* (90 and 86.7%) followed by *T. divericata* (90 and 86.7%) at 100% concentration in both the tested samples. The incidence of the pathogen was reduced by *T.*

*divericata* (26.7 and 23.3%) at 100% concentration followed by *P. hysterothorus* (30 and 33.3%) at same concentration as compared to check (60 and 63.3%) in both the samples. The per cent control of the pathogen was shown by *T. divericata* (55.6 and 63.5%) followed by *P. hysterothorus* (50 and 46.8%) at 100% concentration (Table 1).

**Table 1: *in vitro* effects on seed treatment of leaf extracts on seed germination, incidence and control of *Xanthomonas axanopodis* pv. *vesicatoria* in chilli on SBM**

S. No.	Leaf extracts used	Conc. (%) (w/v)	SEED SAMPLES ON SBM					
			Ca-1227			Ca-1234		
			Seed germination (%)	Incidence of pathogen (%)	Control of pathogen (%)	Seed germination (%)	Incidence of pathogen (%)	Control of pathogen (%)
1.	Check	-	76.7 (61.14)	60.0 (50.77)	0(0.00)	66.7(54.76)	63.3(52.71)	0(0.00)
2.	<i>Clorodendrum inerme</i>	100	76.7 (61.14)	56.7 (48.85)	5.6(13.69)	70.0(56.79)	53.3(46.89)	16.7(24.12)
		30	76.7 (61.14)	56.7 (48.85)	5.6(13.69)	66.7(54.76)	56.7(48.85)	10.3(18.72)
3.	<i>Withinia somnifera</i>	100	80.0 (63.44)	53.3 (46.89)	11.1(19.46)	76.7(61.14)	43.3(41.15)	30.9(33.77)
		30	80.0 (63.44)	56.7 (48.85)	5.6 (13.69)	70.0 (56.79)	46.7 (43.11)	25.4 (30.26)
4.	<i>Lantana camara</i>	100	76.7 (61.14)	53.3 (46.89)	11.1(19.46)	76.7 (61.14)	53.3 (46.89)	15.1 (22.87)
		30	76.7 (61.14)	56.7 (48.85)	5.6 (13.69)	76.7 (61.14)	53.3 (46.89)	14.3(22.22)
5.	<i>Tabernaemontana divericata</i>	100	90.0** (71.56)	26.7** (31.11)	55.6** (48.22)	86.7** (68.61)	23.3** (28.86)	63.5** (52.83)
		30	90.0** (71.56)	33.3** (35.24)	44.4** (41.78)	80.0(63.44)	33.3** (35.24)	47.6** (43.62)
6.	<i>Argimone mexicane</i>	100	80.0 (63.44)	46.7 (43.11)	22.2 (28.11)	76.7 (61.14)	46.7 (43.11)	26.9 (31.24)
		30	76.7 (61.14)	50.0 (45.0)	16.7 (24.12)	66.7 (54.76)	56.7 (48.85)	10.3 (18.72)
7.	<i>Catheranthus roseus</i>	100	90.0** (71.56)	33.3 (35.24)	44.4 (41.78)	73.3 (58.89)	43.3 (41.15)	31.7 (34.24)
		30	86.7** (68.61)	36.7 (37.29)	38.9 (38.59)	70.0 (56.79)	43.3 (41.15)	30.9 (33.77)
8.	<i>Parthenium hysterothorus</i>	100	90.0** (71.56)	30.0** (33.21)	50.0** (45.0)	86.7** (68.71)	33.3 (35.24)	46.8** (43.17)
		30	86.7 (68.61)	36.7 (37.29)	38.9 (38.59)	86.7** (68.71)	36.7 (37.29)	42.1** (40.46)
	CD at 5%	-	4.06	5.16	16.83	9.97	10.60	20.76
	CD at 1%	-	5.60	7.11	22.09	13.77	14.61	28.60

Values are the mean of 3 replicates; Values in parentheses are angular transformed values.

In the earlier studies the bio-efficacy of six medicinal plant extracts was tested *in vitro* using filter paper disc assay and seed treatment method against *Pseudomonas syringae* pv. *pisi* in pea. The maximum antibacterial activity was shown by aqueous extract of *A. sativum* (IA=455.98mm<sup>2</sup>) followed by *T. chebula* (IA=415.25mm<sup>2</sup>). Seed treatment with aqueous extract of *A. sativum* improved seed germination (94.6%) as compared to check (56.3%) and control the incidence of the pathogen in seeds (85.5%)<sup>19</sup>.

Seed treatment with these medicinal plants extracts improved seed germination and control of pathogen due to the presence of bioactive compounds<sup>40</sup>. Seed treatment with *T. bellirica* and *A. sativum* showed maximum seed germination and control of *Xanthomonas pisi*<sup>20</sup>. Plant extracts of *T. chebula* is reported to be an antimicrobial, hepatoprotective, anti-inflammatory, immunomodulatory, an antioxidant and adaptogenic<sup>41-44</sup>. The antibacterial activity of 30 leaf extracts against 11 strains of *X. campestris* pv. *mangiferaeindicae* tried out of them 12 leaf extracts showed antibacterial activity remaining 18 leaf extracts had not shown any inhibitory effect. *Terminalia thorelii* followed by *Azadirachta indica*, *Callistemon rigidus*, *Butea monosperma*, *Calistemon rigidus*, *Capsicum annum*, *Caesalpinia pulcherima*, *Datura inoxia*, *Dolichandrone falcate*, *Holoptelea integrifolia*, *Lantana camera*, *Lawsonia inermis* and *Vitex negundo* also showed good antibacterial activity. In another study, 2300 plant species screened to know their antibacterial activity against the bacteria like

*Escherichia coli* and *Staphylococcus aureus*<sup>45</sup>. Pawar (1999) has screened 110 leaf extracts, 09 root extracts, 36 fruit extracts, 05 stem extracts, 10 seed extracts, 04 bark extracts, 08 gum and 06 latex against 05 bacterial phytopathogens<sup>46</sup>. Aqueous extracts of leaves of *Tamarindus indica* showed good antibacterial activity gainst Gram positive bacteria and hydroalcoholic extracts of leaves in Gram negative bacteria<sup>47</sup> and have wide range of antibacterial activity against gram positive and gram negative bacterial strains<sup>48</sup>. Hot water extract of garlic showed good inhibitory effect against *Xanthomonas citri* and *Erwinia carotovora* and human pathogens<sup>49</sup>. In this study, the improvement in seed germination was shown by all the tested aqueous plant extracts in seed treatment method (Table 2). The relative percent seed germination by plant extracts was as follows- *T. divaricata* > *P. hysterothorus* > *C. roseus* > *A. maxicana* = *W. somnifera* > *L. camara* = *C. inerme*

The highest percentage seed germination showed by *T. divaricata*, *P. hysterothorus* and *C. roseus* at 100% concentration as compared to control (76.7%). There was no improvement in germination by the leaf extract of *L. camara* and *C. inerme* after seed treatment. The relative percent control of the pathogen was as follows-

*T. divaricata* > *P. hysterothorus* > *C. roseus* > *A. maxicana* > *W. somnifera* > *L. camara* > *C. inerme*

Seven aqueous leaf extracts were tested at three different concentrations (25, 50 and 100%) using filter paper disc assay method or seeded agar method against the bacterial

colonies of XAV. The leaf extract showed potential activity against bacterial pathogen as compare to check (Fig. 1). Bacterial blight was more effectively controlled by the water and methanol extracts of *Vitex negundo* than the other plant extracts<sup>50</sup>. The methanolic leaf extracts of *Acacia nilotica*, *Sida cordifolia*, *Tinospora cordifolia*, *Withania somnifera* and *Ziziphus mauritiana* against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Xanthomonas axonopodis* pv. *malvacearum* has been reported<sup>51</sup>. The aqueous extracts of garlic, clove and onion were found effective against *X. axonopodis* pv. *vignaradiatae* during foliar application<sup>52</sup>. *Terminellia chebula* showed an inhibitory effect against *Xanthomonas campestris* pv. *citri*<sup>41</sup>. The plant extracts of *Allium cepa*, *Azadirachta indica*, *Tamarix aphylla*, *Vernonia anthelmentica*, *Plumbago zelanicum*, and *Tegetis erecta* showed significant antibacterial activity at 50% concentration against *X. campestris* pv. *campestris* *in vitro* and showed improved seed germination and as compare to streptomycin<sup>53</sup>.

In the present study the aqueous leaf extracts of *P. hysterophorus* and *L. camara* individually were found most effective against the pathogen at 100% concentration in filterpaper and seeded agar method as compared to check. Seven plant leaf extracts were used and leaf extract of *P. hysterophorus* at 100% concentration showed the highest inhibition zone and activity index was 28.67 mm and 3.85 followed by *L. camara* being 15.33 mm and 1.92 respectively against the pathogen in filter paper and seeded agar method.

**Table 2: In vitro evaluation of antibacterial activity of some plant extracts against seed-borne bacterium (*Xanthomonas axonopodis* pv. *vesicatoria*) in chilli on filter paper method and seeded agar method**

	Leaf extracts	Concentration (w/v)	Test Bacterium	
			IZ (mm)	AI
1.	<i>Lantana camara</i>	100	15.33	1.92
		50	13.33	1.67
		25	11.33	1.42
2.	<i>Clerodendrum inerme</i>	100	11.33	1.42
		50	10.00	1.25
		25	9.33	1.17
3.	<i>Tabernaemontana divericata</i>	100	13.67	1.71
		50	11.00	1.38
		25	10.33	1.29
4.	<i>Parthenium hysterophorus</i>	100	28.67	3.58
		50	24.33	3.04
		25	16.67	2.08
5.	<i>Withania somnifera</i>	100	12.67	1.58
		50	11.00	1.38
		25	09.00	1.13
6.	<i>Argimone mexicane</i>	100	11.00	1.38
		50	10.33	1.29
		25	09.33	1.17
7.	<i>Catheranthus roseus</i>	100	11.66	1.46
		50	10.33	1.29
		25	10.33	1.25

\* Diameter of filter paper (well) disc (8 mm) included inhibition zone in check.



**Fig 1: In vitro control of *Xanthomonas axonopodis* pv. *vesicatoria* using leaf extracts in seeded agar method (note the zone of inhibition)**

#### 4. CONCLUSION

The present study revealed that among seven different plant leaf extracts (aqueous) used for their antibacterial activity, *Parthenium hysterophorus* and *Lantana camara* on filter paper method and seeded agar method gave the promising results against *Xav* causing bacterial leaf spot disease of chilli *in vitro*. In standard blotter method *Tabernaemontana divericata* and *Parthenium hysterophorus* found most effective at 100% concentration. The treatment also improved seed germination and control of pathogen as compared to check.

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