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BIOLOGICAL CONTROL OF SEED-BORNE BACTERIUM *XANTHOMONAS AXONOPODIS* PV. *VESICATORA* (DOIDGE) VAUTERIN, HOSTE, KERSTERS & SWINGS IN BRINJAL USING PLANT EXTRACTS

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Brinjal **(***Solanum melongena* L.) is an important vegetable crop of Solanaceae grown throughout the world. Leaf spot and stem canker disease of brinjal is caused by *Xanthomonas axonopodis* pv. *vesicatoria* (XAV) in the major growing areas of India. Leaf extract of approx 31 medicinal plants at various concentrations (100, 50 and 25%) were tried to know their antimicrobial activities against XAV in using filter paper disc assay, seeded agar and seed treatment methods. In this study aqueous leaf extracts of *Cassia fistula* and *Catharanthus roseus* (47.71% and 39.96%) at 25% concentration followed by *Lawsonia inermis* and *C. roseus* (30.98% and 40.15%) at 100% and 50% concentration were found most effective against the pathogen in standard blotter method as compared to check. The high inhibition zone (IZ) and activity index (AI) was shown by *Saraca asoca* (100%) followed by *Withinia somnifera* (50%) and *Lantana camara* (25%) in filter paper disc assay and seeded agar methods. The present study showed these botanicals effective against the pathogen or reduction in the disease incidence and improves the seed germination significantly.

Key words: Anti-bacterial activity, Filter paper disc assay, Leaf extracts activity, Seeded agar method, Standard blotter method.

Introduction

Brinjal **(***Solanum melongena* L.) or eggplant (Kings of vegetables) or aubergine (French name) of Solanaceae is one of the most common, popular and principal vegetable crop grown worldwide. It is consumed and grown in warm areas intensively in India, Bangladesh, Pakistan, China, Philippines, Egypt, France, Italy and Unite States. In India, major brinjal producing states are West Bengal, Orissa, Bihar, Gujarat, Maharashtra, Karnataka, Uttar Pradesh, Andhra Pradesh and Rajasthan. In Rajasthan, Alwar, Kota, Jaipur, Sriganganagar and Bharatpur are the major producing districts[1].

Brinjal contain large amounts of free reducing sugars, anthocyanin, phenols, glycoalkaloids (as solasodine), dry matter and amide proteins. In Brazil, eggplant is consumed extensively and believed that

infusion of a powdered preparation of the fruit may reduce serum cholesterol. The composition of brinjal (per 100g of edible portion) reported as moisture (92.7 g), Sodium (3.0 mg), protein (1.4g), Potassium (2.0 mg), fat (0.3 g), Copper (0.17mg), minerals (0.3 g), Sulphur (44.0 mg), fibre (1.3g), Chlorine (52.0 mg), carbohydrates (4.0g), vitamin A (124LU), Calcium (18mg), Thiamine (0.04 mg), Magnesium (16 mg), Oxalic acid (18 mg), Nicotinic acid (0.09 mg), Phosphorus (47mg), Vitamin C (12.0 mg) and Iron (0.9 mg)[2-4].

Xanthomonas axonopodis pv. *vesicatora* (Doidge) Dye (syn: *X. campestris* pv. *vesicatoria*) (XAV) produce spot on leaf and cankers on stem is a Gram's negative aerobic rods with single polar flagellum. On Tween-80 medium the bacterium is appeared as circular, raised, yellow colonies surrounded by zone of white crystals of calcium salt of fatty acids released from tween by lipolytic enzymes and reported to be seed-borne in brinjal[5-8].

Material and Methods

Collection of plant materials

Plant leaves of selected eleven plants were collected from Jaipur, Rajasthan, India for this experiment. These medicinal plants leaf extracts *viz.* Mexican prickly poppy (peeli kataili, *Argemone mexicana* L.) of family Papaveraceae, Madagascar periwinkle (sadabahar, *Catharanthus roseus*) of Apocynaceae, Lantana (Kuri, *Lantana camara*) of Verbenaceae, withania (ashwagandha, *Withania somnifera*) of Solanaceae, prickly chaff flower (apamarga, *Achyranthes aspera*) of Amaranthaceae, Indian laburnum (amalats, *Cassia fistula*) of Caesalpiniaceae, billygoat-weed (Jangli pudina, *Ageratum conyzoides*) of Asteraceae, Varigated Bauhinia (Kachnar, *Bauhinia varigata*) of Fabaceae, Tree Mignonette (Mehandi/ Henna *Lawsonia inermis*) of *Lythraceae,* Holy Basil (Tulsi, *Ocimum sanctum*) of Lamiaceae, Indian lilac or margosa (neem, *Azadirachta indica*) of Meliaceae were tested *in vitro* for control of pathogen in standard blotter method (Table 1). For the experiments 10 g fresh leaves of each plant was taken, washed thoroughly, surface sterilized and 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 filtered extract was treated as stock solution.

In filter paper disc method and seeded agar method 31 leaves extracts *viz. Withania somnifera* (L) Dunal (Solanaceae), *A. mexicana* L. (Papaveraceae), *Ageratum conyzoides* L. (Asteraceae), *Lawsonia inermis* L. (Lythraceae), *Azadirachta indica* A. Juss. (Meliaceae), *Catharanthus roseus* (L.) G. Don. (Apocynaceae), *Lantana camara* L. (Verbenaceae), *Cassia fistula* L. (Fabaceae), *Bryophyllum piinnatum* (Lam.) Oken (Crassulaceae), *Barleria cristata* L. (Acanthaceae), *Achyranthus aspera* L. (Amaranthaceae), *Bauhinia verigata* L. (Fabaceae- Caesalpinioideae), *Euphorbia stenoclada Baill*. (Euphorbaceae), *Thevesia peruviana* (pers). K. Shum (Apocynaceae), *Saraca asoca* (Roxb.) Willd. (Fabaceae), *Opuntia stricta* Haw. (Haw.) (Cactaceae), *Oxalis griffithii* Edgew.&Hook. F.(Oxalidaceae), *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms (Menispermaceae), *Nirium indicum* L. (Apocynaceae), *Thevetia nerifolia* Juss. (Apocynaceae), *Amaranthus spinosus* L. (Amaranthaceae), *Phyllanthus emblica* L. (Euphorbiaceae), *Parthenium hysterophorus* L. (Asteraceae), *Ficus religiosa* L. (Moraceae), *Dracaena marginata* (Asperagaceae), *Crinum latifolium* L. (Amaryllidaceae), *Holoptelea integrifolia* (Roxb.) Planch (Ulmaceae),

Aegle marmelos (L.) Correa. (Rutaceae), *Aloe vera* (L.)Burm.f *(*Asphodelaceae), *Asparagus racemosus* Willd. (Asperagaceae) and *Boerhavia diffusa* L (Nyctaginaceae) were tried[9].

Three methods namely seed treatment in Standard blotter method; filter paper disc assay and seeded agar method were tried for the study. Two seed samples of brinjal SM 001 and SM 035 naturally infected with XAV were treated individually with the aqueous leaf extracts of 11 medicinal plants for 4 hrs in two different concentrations, pure (100%, w/v) and diluted (50 and 25% v/v) in triplicate (100 seeds/ sample). Percent seed germination, seedling symptom, incidence and inhibition of the bacteria calculated by following formula[9-10]

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

In another 2 methods namely seeded agar method and filter paper disc method (disc diffusion method) antibacterial activity of 31 leaf extracts against XAV was also carried out[**11-13**]. Filter paper discs of 8 mm diameter impregnated with plant extracts were placed in the inoculated plates and 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 inhibition zones were measured and inhibition annulus was calculated by following formula[14,15]

[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[7,8,16-18].

Result and discussion

Recently researchers showed much interest in non-hazardous eco-friendly management of various microbes or pests using medicinal plants or biopesticides[19,20]. Derivatives of these plants are good source of agrochemicals, bioactive compounds as alkaloids, flavonoids, tannins, saponins and terpenoids[21-27]. Leaf extracts of various plants are known to possess antimicrobial activity to control of various plant diseases[28-30].

In the present study, aqueous leaf extracts of 11 medicinal plants were tried and found effective to control the pathogen XAV. The improvement in seed germination was shown by all the tested leaf extracts as compared to infected seeds. The maximum improvement was shown by *Cassia fistula* and *Catharanthus roseus* (71.5% and 79.4%) at 25% in acc nos SM001 and SM035 followed by *Lawsonia inermis* and *C. roseus* (70.2% and 79.2%) at 100% and 50% in acc nos SM001 and SM035 respectively as compared to check (57.8% and 61.2%). The incidence of the pathogen was reduced by *Cassia fistula* and *C. roseus* (29.7% and 30.2%) at 25% concentration followed by *L. inermis* and *C. roseus* (50.1% and 30.2) at 100% and 50% concentration as compared to check (56.8% and 50.8%) in both the samples respectively. The maximum percent control of the pathogen was shown by *Cassia fistula* and *C. roseus* (47.71% and 39.96%) at 25% concentration followed by *L. inermis* and *C. roseus* (43.13% and 40.15%) at 100% and 50% concentration respectively (Table-1).

Bio-efficacy of 6 plant extracts was tested *in vitro* using filter paper disc assay and seed treatment method against *Pseudomonas syringae* pv. *pisi* in pea. Maximum antibacterial activity and improved seed germination was shown by aqueous extract of *Allium sativum* followed by *T. chebula*[15]. The plant extracts of *A. cepa*, *Azadirachta*

S. No.	Leaf extracts used	Conc. (%) (w/v)	SEED SAMPLES					
			SM001			SM035		
			Seed germination (%)	Incidence of pathogen (%)	Control of pathogen (%)	Seed germination (%)	Incidence of pathogen (%)	Control of pathogen (%)
	Check		57.8	56.8	0	61.2	50.8	0
1.	*Argemone maxicana*	25	66.2	46.7	17.78	63.8	48.7	04.13
		50	65.4	42.9	24.47	62.3	48.9	03.74
		100	64.7	40.7	28.34	60.5	45.5	10.43
2.	*Achyranthes aspera*	25	63.4	49.7	12.50	63.4	39.2	22.83
		50	64.2	47.6	16.19	66.1	35.2	30.70
		100	65.3	44.3	22.00	69.7	33.3	34.44
3.	*Cassia fistula*	25	71.5	29.7	47.71	68.8	33.2	34.64
		50	68.8	31.3	44.89	63.4	39.2	22.83
		100	67.4	37.6	33.80	60.1	41.9	17.51
4.	*Ageratum conyzoides*	25	64.4	40.9	27.99	61.2	43.0	15.35
		50	63.2	41.3	27.28	63.4	39.2	22.83
		100	62.3	43.6	23.23	65.5	35.5	30.11
5.	*Bauhinia varigata*	25	65.6	42.7	24.82	66.1	41.0	19.29
		50	62.3	45.5	19.89	66.3	37.2	26.77
		100	66.7	44.0	22.53	65.5	34.6	31.88
6.	*Catharanthus roseus*	25	60.2	47.5	16.37	79.4	30.2	39.96
		50	63.4	43.2	23.94	79.2	30.4	40.15
		100	66.7	41.7	36.58	74.8	34.2	32.67
7.	*Lawsonia inermis*	25	59.7	39.2	30.98	60.1	36.2	28.74
		50	62.7	50.1	11.79	58.4	49.8	02.00
		100	70.2	32.3	43.13	59.4	40.6	20.07
8.	*Ocimum sanctum*	25	61.8	55.4	02.46	60.1	49.8	02.00
		50	60.1	50.8	10.56	58.4	48.8	02.00
		100	58.3	52.2	08.09	58.7	48.8	03.93
9.	*Withania somnifera*	25	58.4	25.4	55.28	58.4	49.8	03.93
		50	59.2	38.7	31.86	56.9	52.3	02.00
		100	61.5	36.2	36.26	55.3	42.6	16.14
10.	*Lantana camara*	25	69.5	37.7	33.62	69.5	37.6	25.98
		50	71.5	42.1	25.88	69.7	40.6	20.07
		100	68.4	46.4	00.70	70.5	49.4	02.75
11.	*Azadirachta indica*	25	60.1	45.5	19.89	60.1	49.4	02.75
		50	61.2	42.5	25.17	58.4	49.8	02.00
		100	67.2	40.7	28.34	56.7	48.8	03.93

Table-1: *In vitro* seed treatment by leaf extracts on seed infected with *Xanthomonas axonopodis* pv. *vesicatoria* in brinjal

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 [31].

Seed treated with *Terminalia*

S. No.	Leaf extracts	Con. % (w/v)	*Xanthomonas axonopodis* pv. *vesicatoria*	
			IZ (mm)	AI
1.	*Withania somnifera*	100	26.66	4.44
		50	36.66	6.11
		25	31.66	5.27
2.	*Argemone Mexicana*	100	26.66	4.44
		50	30.00	5.00
		25	35.00	5.83
3.	*Ageratum conyzoides*	100	35.00	5.83
		50	28.33	4.72
		25	31.66	5.27
4.	*Lawsonia inermis*	100	31.66	5.27
		50	28.33	4.72
		25	35.00	5.83
5.	*Azadirachta indica*	100	26.66	4.44
		50	31.66	5.27
		25	35.00	5.83
6.	*Catharanthus roseus*	100	25.00	4.16
		50	26.66	4.44
		25	26.66	4.44
7.	*Lantana camara*	100	30.00	5.00
		50	35.00	5.83
		25	38.33	6.38
8.	*Cassia fistula*	100	25.00	4.16
		50	31.66	5.27
		25	31.66	5.27
9.	*Bryophyllum pinnatum*	100	31.66	5.27
		50	35.00	5.83
		25	35.00	5.83
10.	*Barleria cristata*	100	35.00	5.83
		50	35.00	5.83
		25	31.66	5.21
11.	*Achyranthus aspera*	100	31.66	5.27
		50	33.33	5.55
		25	31.66	5.27
12.	*Bauhinia verigata*	100	36.66	6.11
		50	35.00	5.83
		25	31.66	5.27
13.	*Euphorbia stenoclada*	100	33.33	5.55
		50	30.33	5.05
		25	31.66	5.27
14.	*Thevesia peruviana*	100	35.00	5.83
		50	35.00	5.83
		25	28.33	4.72
15.	*Saraca asoca*	100	38.33	6.38
		50	36.66	6.11
		25	31.66	5.27
16.	*Opuntia stricta*	100	35.00	5.83
		50	33.33	5.55
		25	28.33	4.72
17.	*Oxalis griffithii*	100	31.66	5.27
		50	28.33	4.72
		25	31.66	5.27
18.	*Tinospora cordifolia*	100	35.00	5.83
		50	35.00	5.83
		25	35.00	5.83
19.	*Nirium indicum*	100	35.00	5.83
		50	31.66	5.27
		25	31.66	5.27
20.	*Thevetia nerifolia*	100	35.00	5.83
		50	35.00	5.83
		25	35.00	5.83
21.	*Amaranthus spinosus*	100	28.33	4.72
		50	35.00	4.16
		25	23.33	3.88
22.	*Phyllanthus emblica*	100	25.00	4.16
		50	25.00	4.16
		25	21.66	3.61
23.	*Parthenium hysterophorus*	100	28.33	4.72
		50	23.33	3.88
		25	20.00	3.33
24.	*Ficus religiosa*	100	26.66	4.44
		50	25.00	4.16
		25	25.00	4.16
25.	*Dracaena marginata*	100	28.33	4.72
		50	26.66	4.44
		25	21.66	3.61
26.	*Crinum latifolium*	100	25.00	4.16
		50	21.66	3.61
		25	23.33	3.88
27.	*Holoptelea integrifolia*	100	28.33	4.72
		50	26.66	4.44
		25	23.33	3.88
28.	*Aegli marmelos*	100	31.66	5.27
		50	23.33	3.88
		25	21.66	3.61
29.	*Aloe vera*	100	35.00	5.83
		50	28.33	4.72
		25	23.33	3.88
30.	*Asparagus racemosus*	100	28.33	4.72
		50	25.00	4.16
		25	20.00	3.33
31.	*Boerhavia diffusa*	100	23.33	3.88
		50	25.00	4.16
		25	21.66	4.44

Table-2: *In vitro* evaluation of antibacterial activity of leaf extracts against *Xanthomonas axonopodis* pv. *vesicatoria* in brinjal

bellirica and *A. sativum* showed maximum seed germination and control of *Xanthomonas pisi* [15,32]. About 110 leaf extracts screened, 09 root extracts, 36 fruit extracts, 05 stem extracts, 10 seed extracts, 04 bark extracts, 08 gums and 6 latexes against 05 bacterial phytopathogens. The leaf extracts of several medicinal plants were used to study their antimicrobial strength against *Xanthomonas axonopodis* pv. *vesicatoria* in chilli. 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[33]. Aqueous leaves extract of *Tamarindus indica* showed good antibacterial activity against Gram positive bacteria and hydro-alcoholic extracts in Gram negative bacteria[34] and wide range of antibacterial activity against bacterial strains[35]. The improvement in seed germination was shown by all the tested aqueous plant extracts (Table 1). The relative percent seed germination was as follows-

Cassia fistula> *Lantana camara*> *Lawsonia inermis*> *Azadirachta indica*> *Argemone mexicana*> *Bauhinia varigata*> *Ageratum conyzoides*> *Achyranthes aspera* > *Ocimum sanctum*> *Withania somnifera*> *Catharanthus roseus*>check

In the present study, thirty one leaf extracts (aqueous) were tested using filter paper disc assay method or seeded agar method against the bacterial colonies of XAV. The highest inhibition zone and activity index was shown by extract of *Lantana camara* (38.33 mm and 6.38) at 25% concentration followed by *Withania somnifera* and *Bauhinia* (36.66 mm and 6.11) at 100% concentration on against *X. A.* pv. *vesicatoria* (Table-2, Fig.1).

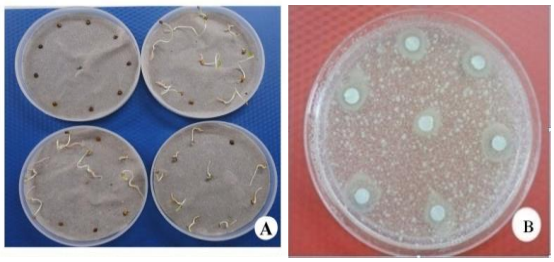

Fig. 1 A- Control of *Xanthomonas axonopodis* pv. *vesicatoria* by using extracts as check, *Cassia fistula, Catharanthus roseus* and *Lawsonia innermis* (clockwise) showing improvement in seed germination in treated seeds, **B.** Control of XAV using leaf extract of *Lantana camara* at 25% concentration in seeded agar.

The maximum activity index in filter paper disc assay was shown by *Saraca asoca* (100%), *W. somnifera* (50%) and *L. camara* (25%) at various concentrations. At 100% concentration in filter paper disc assay the activity index was found maximum by *S. asoca* followed by *Boerhavia diffusa.* At 50% concentration *W. somnifera* showed maximum activity index followed by *Barleria cristata, Thevesia peruviana* and *Achyanthus aspra.* At 25% concentration effective activity index was shown by *L. camara* and minimum by *B. diffusa.*

Bacterial blight in rice was effectively controlled by water and methanol extracts of *Vitex negundo* than the other plant extracts[36]. The methanolic leaf extracts of various medicinal plant extracts against human pathogenic bacteria and phytobacteria as *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* and *Xanthomonas axonopodis* pv. *malvacearum* were found effective[37]. The aqueous extracts of garlic, clove and onion were found effective against *X. a.* pv. *vignaradiatae* in foliar application[38]. *Terminellia chebula* showed an inhibitory effect against *X. campestris* pv. *citri* [39].

In the present study the seven aqueous leaf extracts *Cassia fistula* and *C. roseus* (47.71% and 39.96%) at 25% concentration followed by *Lawsonia inermis* and *C. roseus* (30.98% and 40.15%) at 100% and 50% concentration were found most effective against the pathogen in seed treatment in standard blotter method as compared to check. Out of 31 leaf extracts, *L. camara* (38.33 mm and 6.38) at 25% concentration followed by *W. somnifera* (36.66 mm and 6.11) at 50% concentration and *Saraca asoca* (38.33 mm and 6.38) at 100% concentration were found most effective to control the pathogen.

Conclusion

The present study revealed that among eleven different plant leaf extracts (aqueous) used for their antibacterial activity against the pathogen in standard blotter method and aqueous leaf extracts *C. fistula* and *Catharanthus roseus* (47.71% and 39.96%) at 25% concentration followed by *Lawsonia inermis* and *C. roseus* (30.98% and 40.15%) at 100% and 50% concentration were found most effective. The treatment also improved seed germination and control of pathogen as compared to check. In filter paper disc assay *Lantana camara* (38.33 mm and 6.38) at 25% concentration followed by *W. somnifera* (36.66 mm and 6.11) at 50% concentration and *Saraca asoca* (38.33 mm and 6.38) at 100% concentration were found most effective to control the pathogen.

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