

Bioactivity of Aqueous Extracts of Leaves and Fruits against *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Dowson in Black Gram (*Vigna mungo*)

Vinod Kumar Jain¹, Dilip Kumar Sharma^{2*} and Kailash Agrawal³

¹Department of Botany, Poddar International College, Jaipur

²Vardhman Mahaveer Open University (VMOU), Kota

³Dean, Faculty of Basic & Applied Sciences, Vivekananda Global University, Jaipur

*Corresponding Author E-mail: drdilipsharma12@gmail.com

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ABSTRACT

For the control of halo blight disease caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Dowson in black gram, about 30 plant extracts were used. Among them is an aqueous extract of leaves of *Moringa oleifera* followed by *Withania somnifera*, *Lantana camara*, *Datura stramonium* and *Hibiscus rosa-sinensis* and fruits of *Citrus aurantifolia* followed by *Embllica officinalis* at 100% concentration gave better inhibition of the pathogens; significantly ($P \geq 0.01\%$) improved seed germination and reduced the incidence of the pathogens in both filter paper disc assay and seed treatment method (Standard Blotter Method, SBM).

Keywords: Black Gram, *Hibiscus*, *Withania somnifera*, *Pseudomonas*.

INTRODUCTION

Black gram (*Vigna mungo*), commonly known as urd bean, belongs to the pulses family Fabaceae. It is grown on about 3.1 million hectares, producing 1.49 million tones, accounting for 13% of India's pulse production. Rajasthan, Maharashtra, Madhya Pradesh, Tamil Nadu, Uttar Pradesh, Andhra Pradesh, and West Bengal are the major producers of black gram in India. It is grown during Kharif, but in some states, such as Andhra Pradesh and West Bengal, it also grows as a rabi crop. In Rajasthan, it is grown on 112.8 thousand hectares producing 3250

thousand tones. The primary growing districts are Dungarpur, Bhilwara, Chittorgarh, Banswara, Bundi, Jhalawar, Kota, Pratapgarh, and Udaipur. Rajasthan state accounts for 22% of India's total black gram production (Jeswani & Baldev, 1997, Jansen, 2006, & Anonymous, 2021 a & b).

The halo blight disease is incited by *Pseudomonas syringae* pv. *phaseolicola*. The disease appeared on leaves as in form of water-soaked spots and greasy, halo of variable and may not be produced during high temperatures.

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On pods, symptoms appear as small, circular, water-soaked lesions or streaks appeared. Bacterial oozing is light cream or silver in color and is frequently linked with these patches. Discoloration, shrivelling, and bacterial contamination of seeds are common symptoms of pod infection; nevertheless, some seeds may appear to be healthy (Taylor, Dudley & Persly, 1979, Singh, Gurha & Ghosh, 1998).

MATERIALS AND METHODS

Seed samples of black gram naturally infected with *Pseudomonas syringae* pv. *phaseolicola* individually with 90 and 100% incidence (ac. nos. Vm-1107 and Vm-1122) were selected to study the control of the pathogens by using

filter paper disc assay method and seed treatment method.

(i) Filter paper disc assay method

The sterilized Whatman filter paper discs of 5 mm size impregnated in extract of leaves and fruits were placed in seeded agar plates prepared with the pathogen. The discs are soaked with sterilized distilled water and incubated at $30 \pm 2^\circ\text{C}$ for 3-5 days. Inhibition annulus (mm^2) was calculated and used to compare the antibacterial activity of the test plant extracts (Thornberry, 1959). The clearing around the discs was recorded daily up to 5 days at 24 hrs intervals. The inhibition annulus was calculated by the following formula (Thornberry, 1959, Smale & Keil, 1966).

$$\text{Inhibition Annulus (IA)} = \pi (R_1 + R_2) (R_1 - R_2)$$

Where R_1 = radius of filter paper disc + Radius of inhibition zone

R_2 = Radius of filter paper disc

$$\pi = 3.14$$

(ii) Seed treatment method

Naturally infected seed samples of the pathogens were soaked in plant extracts individually for 1 hr. Seeds soaked in distilled water served as check. All the seeds (treated) were incubated for 7 days by SBM (standard

blotter method) (Anonymous, 1985). Percent seed germination, symptomatic seedlings and incidence of the respective pathogen were recorded. The following formula was used for calculate the Percent control-

$$\text{Percent control} = \frac{\text{Incidence in check (C)} - \text{Incidence in treatment (T)}}{\text{Incidence in check (C)}} \times 100$$

RESULTS AND DISCUSSION

(i) Filter paper disc assay method

For the control of *P. syringae* pv. *phaseolicola* 5 fresh leaves and 2 fresh fruits effective extracts were evaluated in this method. The highest inhibition annulus against *P. syringae* pv. *phaseolicola* were shown by leaves extracts of *Moringa oleifera* (595.8 mm^2 and 734.8 mm^2), followed by *Withania somnifera* (432.5 mm^2 and 395.6 mm^2), *Lantana camara* (326.6 mm^2 and 207.2 mm^2), *Datura stramonium* (263.8 mm^2 and 134.2 mm^2) and *Hibiscus rosa-sinensis* (134.2 mm^2 and 75.4 mm^2) at 100% conc. as compared to check in centers in ac. nos. Vm-1107 and Vm-1122

respectively.

The highest inhibition annulus against *P. syringae* pv. *phaseolicola* were shown by extracts of fruits of *Citrus aurantifolia* (640.6 mm^2 and 686.9 mm^2), followed by *Embllica officinalis* (326.6 mm^2 and 294.4 mm^2) at 100% concentration in ac. nos. Vm-1107 and Vm-1122 respectively.

(II) Seed treatment method

The percent control of the pathogen was shown maximum of leaves extract of *Moringa oleifera* (77.8 and 76.0%) followed by *Withania somnifera* (63.0 and 64.0%), *Lantana camara* (55.6 and 60%), *Datura stramonium* (51.9 and 43.9%) and *Hibiscus*

rosa-sinensis (48.1 and 52.0%) and fruits extract of *Citrus aurantifolia* (70.3 and 80%) and *Embllica officinalis* (59.2 and 55.9%), at 100% conc. as compared to check in ac. nos. Vm-1107 and Vm-1122 respectively.

Among all the leaf extracts used *Moringa oleifera* (93.3 and 96.7%), followed by *Withania somnifera* (73.3 and 86.7%), *Lantana camara* (73.3 and 80%), *Datura stramonium* (66.7 and 70%) and *Hibiscus rosa-sinensis* (60 and 63.3%) at 100% conc. showed the significant ($P \geq 0.01\%$) germination of seeds after treatment as compared to check (30 and 33.3%) in ac. nos. Vm-1107 and Vm-1122. Infected seeds treated with extract of fruits of *Citrus aurantifolia* (86.7 and 93.3%) and *Embllica officinalis* (80 and 76.7%) showed significant seed germination.

In the present study, the pathogen was significantly inhibited by leaf extract of *Moringa oleifera* and fruit extract of *Citrus aurantifolia* and the treatment improved seed germination. This may be due to the presence of bioactive compounds in them. *Moringa oleifera* plant has antibacterial compounds

namely benzyl isothiocyanate and niazimician and *Citrus aurantifolia* have limonene, citral, erucylamide and methoprene.

Seven partially purified plant extracts viz. mahua flowers, satyanashi leaves, bitter temru fruits, neem gloy stems, amaltas leaves, trichoma pods and custard apple seeds were tested against bacterial plant pathogens (*P. solenacearum*) by disc diffusion technique. Product components from mahua flowers and satyanashi were effective against the pathogen at 1000 ppm (Meena, Mali & Choudhary, 2007).

In other studies also, a large number of plant extracts have been reported to be effective against bacterial pathogen *P. syringae* pv. *syringae* (Yadav & Nath, 2006, Sharma, 2006, Jain, 2009, Anand, 2009, & Bakshi, 2009), *Xanthomonas pisi* (Verma & Agrawal, 2017), *Pseudomonas syringae* pv. *pisi* (Verma and Agrawal, 2015), *Xanthomonas axonopodis* pv. *vesicatoria* (Sharma, 2016, 2018) and *Xanthomonas campestris* pv. *cucurbiatae* (Arora & Sharma, 2021).

Table 1: In vitro evaluation of aqueous plant part extracts for inhibition of *Pseudomonas syringae* pv. *phaseolicola* using filter paper disc assay in black gram (*Vigna mungo*)

S. No.	Plants extracts used ↓	Seed samples							
		Ac. no. Vm-1107				Ac. no. Vm-1122			
	Conc. (w/v) →	50%		100%		50%		100%	
		R _{IZ} (mm)	IA (mm ²)**	R _{IZ} (mm)	IA (mm ²)**	R _{IZ} (mm)	IA (mm ²)**	R _{IZ} (mm)	IA (mm ²)**
1.	Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2.	<i>Datura stramonium</i>	3.5	30.6	9.5	263.8	3.5	30.6	7.0	134.2
3.	<i>Hibiscus rosa Sinensis</i>	4.5	44.0	7.0	134.2	3.0	8.6	5.5	75.4
4.	<i>Lantana camara</i>	4.0	18.8	10.5	326.6	4.5	43.1	8.5	207.2
5.	<i>Moringa oleifera</i>	7.0	134.2	14.0	595.8	8.0	95.0	15.5	734.8
6.	<i>Withania somnifera</i>	5.5	75.4	12.0	432.5	5.0	58.9	11.5	395.6
7.	<i>Citrus aurantifolia</i>	7.5	157.0	14.5	640.6	8.0	95.0	15.0	686.9
8.	<i>Embllica officinalis</i>	5.0	58.9	10.5	326.6	4.5	44.0	10.0	294.4
	S.Em.	3.4344		0.9539		1.5469		1.4230	
	CD at 5%	10.392		2.886		4.680		4.305	
	CD at 1%	14.471		4.019		6.518		5.996	

Values are the mean of 3 replicates; R_{IZ} = radius of inhibition zone; IA = inhibition annulus; ** = significant at 1% ($P \geq 0.01$)

CONCLUSIONS

Worldwide, the black gram halo blight is a dangerous disease. About 30 plant extracts

were tested in this investigation to manage the disease. Out of them, leaf extracts of *Moringa oleifera*, followed by *Withania somnifera*,

Lantana camara, *Datura stramonium*, and *Hibiscus rosa-sinensis*; and fruits of *Citrus aurantifolia*, followed by *Emblica officinalis* at 100% concentration, gave better inhibition of the *Pseudomonas syringae* pv. *phaseolicola*, improved seed germination and reduced the incidence of the pathogens. This study could be useful to businesses and industries engaged in non-hazardous. For those working in the disciplines of seed technology and management, particularly those working in plant pathology, Additional research is needed to understand the process of antibiosis in greater detail.

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The author declares no conflict of interest.

Author Contribution:

All authors contributed equally to establishing the research and design experiment topic.

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