

Research Article



***In vitro* Evaluation of Antibacterial Activity of Some Medicinal Plants against *Xanthomonas pisi* Causing Leaf Spot of Pea**

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ABSTRACT

The plant extracts of eighteen botanicals were used to study their antibacterial efficacy against *Xanthomonas pisi*, causal agent of leaf spot disease in pea. Plant extracts at two concentrations 50% and 100% were used to evaluate their antibacterial effects on studied pathogen using filter paper disc assay and seed treatment methods. Among eighteen medicinal plants, aqueous extracts of *Terminalia bellirica* (Fruit), *Allium sativum* (Bulb), *Allium cepa* (Bulb), *Terminalia chebula* (Fruit), *Emblca officinalis* (Fruit), *Mentha piperata* (Leaf), *Azadirachta indica* (Leaf) and *Tamarindus indica* (Fruit) showed maximum inhibitory effects against the studied pathogen. The maximum seed germination and control of the pathogen was observed by seed treatment with aqueous extract of *Terminellia bellirica* (95% and 88.2%) and *Allium sativum* (90.6% and 81.2%) at 100% concentration. The present study indicates that leaf spot disease of pea can be effectively controlled by eco-friendly, economical and nonhazardous manner.

Keywords: Antibacterial efficacy, Control, leaf spot, Pea, Plant extracts, *Xanthomonas pisi*.

INTRODUCTION

Bacterial leaf spot caused by *Xanthomonas pisi* (ex Goto and Okabe, 1958)¹ is an important seed-borne disease which frequently causes considerable reduction in yield and quality of the pea and economical losses to the growers. There is increasing interest in identifying effective novel biological methods to control plant pathogens². Plant extracts have been found effective against a wide range of disease pathogens. The bio-efficacy of plant parts extracts are often systemic, specific, economical and environment friendly in nature³. Plant extracts have played significant role to manage pathogens and to improve yield and quality production. The aims of this study were to evaluate antibacterial efficacy of some selected plant parts extracts against the bacterial pathogen *Xanthomonas pisi* causing leaf spot disease of pea. In the present study, extracts of eighteen plant parts were tested to reduce the disease incidence under *in vitro* condition.

MATERIALS AND METHODS

Collection of plant materials

Plant parts of selected eighteen plants (Table 1) were collected in and around Jaipur, Rajasthan, India. The plant parts were brought to laboratory and washed with tap water and then with double sterile water. Plant parts were shade dried for 3 days and then oven dried at 40°C for 10 h. The oven dried plant parts were powdered and passed through 40 mesh sieve.

Preparation of plant extracts

The dried powdery plant parts boiled in sterile double distilled water at the rate of 10g plant parts powder in 10 ml of water (1:1 w/v) and filtered through double layered sterilized cheese cloth. The filtrate was used as the plant extract of 100% conc. Further dilution of 50% conc. was made with sterile distilled water.

Filter paper disc assay method

Antibacterial activity assay against *X. pisi* were carried out using disc diffusion method⁴. Bacterial suspensions (10 ml) of tested bacteria were spread by a sterile swab on nutrient agar medium. Filter paper discs (6 mm diameter) were impregnated with plant extracts and placed in the inoculated plates. Filter paper discs soaked in double sterile distilled water were used as check, placed in the middle of the plates. The plates were incubated at 30±2° C for 48 h. The inhibition zones were measured in diameter (mm) around of the discs. Inhibition zone (mm) was calculated and used to compare the antibacterial activity of the test plant extracts. The inhibition annulus was calculated by following formula^{5,6}.

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

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

R_2 = Radius of filter paper disc and

π = 3.14

Seed treatment

Seeds of naturally infected samples of the studied pathogen were soaked in various plant extracts individually (at 50% and 100% conc.) for 1h. Seeds soaked



in sterile distilled water served as check. All the treated seeds were incubated for 7 days by standard blotter method⁷. Percent seed germination, symptomatic seedlings and incidence of the respective pathogen were recorded. 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

Results and discussion

In the present study, the use of aqueous plant extracts were found effective to control the bacterial pathogen *Xanthomonas pisi* causing leaf spot disease of pea. In recent years, much interest has been developed to exploit the antibacterial activity of medicinal plants to control

various plant diseases. Plant extracts are eco-friendly and economical sources of new agrochemicals for the control of plant diseases. The biochemical compounds of plant origin are nonphytotoxic, systemic and easily biodegradable^{8,9,10}. It is now known that various plant extracts can reduce populations of pathogens and control disease development. Consequently, they have a potential as environmentally safe alternatives and components in integrated disease management programs¹¹. Hence, plant extracts have been investigated for their antibacterial activity^{12,13}. In the present study, different parts of eighteen aqueous plant extracts were evaluated for their antibacterial activity against bacterial pathogen *X. pisi*.

Table 1: *In vitro* evaluation of some aqueous plant extracts for inhibition of *Xanthomonas pisi* using filter paper disc assay.

Plant extracts Conc. In % (w/v)	Family	Part used	Inhibition Annulus (%) against <i>Xanthomonas pisi</i>	
			50% IA*(mm ²)	100% IA*(mm ²)
<i>Withania somnifera</i>	Solanaceae	Leaves	48.56	128.63
<i>Azadirachta indica</i>	Meliaceae	Leaves	164.61	290.5
<i>Polyalthia longifolia</i>	Annonaceae	Leaves	8.63	54.54
<i>Tamarindus indica</i>	Fabaceae	Fruits	129.87	219.63
<i>Emblca officinalis</i>	Euphorbiaceae	Fruits	93.41	273.38
<i>Allium sativum</i>	Liliaceae	Bulbs	159.83	432.53
<i>Coleus barbatus</i>	Lamiaceae	Leaves	80.96	122.59
<i>Prosopis julifera</i>	Fabaceae	Leaves	34.71	76.39
<i>Acacia catechu</i>	Fabaceae	Fruits	47.68	118.39
<i>Citrus aurantifolia</i>	Rutaceae	Fruits	106.79	226.86
<i>Curcuma longa</i>	Zingiberaceae	Rhizome	18.84	32.13
<i>Mentha piperita</i>	Lamiaceae	Leaves	108.98	273.38
<i>Allium cepa</i>	Liliaceae	Bulb	189.45	440.10
<i>Terminalia bellirica</i>	Combretaceae	Fruits	152.32	483.64
<i>Terminalia chebula</i>	Combretaceae	Fruits	93.41	437.06
<i>Nicotiana tobaccum</i>	Solanaceae	Leaves	22.43	84.55
<i>Ocimum sanctum</i>	Lamiaceae	Leaves	69.57	155.12
<i>Zingiber officinalis</i>	Zingiberaceae	Rhizome	18.84	80.96
S.Em.		±0.25	±034	±0.15
C.D. at 5%		0.72	0.976	0.43
C.D. at 1%		0.973	1.3	0.576

Filter paper disc assay

Eighteen aqueous plant extracts were tested at two different concentrations (50 and 100%) using filter paper disc assay method against the bacterial colonies of *Xanthomonas pisi*. The data revealed that seven aqueous plant extracts namely *Azadirachta indica*, *Emblca officinalis*, *Allium sativum*, *Mentha piperata*, *Allium cepa*, *Terminalia bellirica*, *Terminalia chebula* out of eighteen showed broad spectrum activity against bacterial

pathogen over check (Treated with sterile distilled water). In earlier studies, bacterial blight was more effectively controlled by the water and methanol extracts of *Vitex negundo* than the other plant extracts³. A significant antibacterial activity of methanol 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 reported¹⁴. The



foliar application of aqueous extracts from garlic, clove and onion bulb are effective against bacterial leaf spot

disease caused by *X. axonopodis* pv. *vignaradiatae* of greengram¹⁵.

Table 2: Evaluation of some aqueous plant extracts against *Xanthomonas pisi* in pea on seed treatment using standard blotter method

Plant Extracts Used	Conc. % (w/v)	Seed germination (%)	Control of pathogen (%)	Conc. % (w/v)	Seed germination (%)	Control of pathogen (%)
Check	50	58.3 (49.78)	-	100	58.3 (49.78)	-
<i>Azadirchta indica</i>	50	69.6 (56.54)	56.8 (48.91)	100	79.3 (62.94)	64.7(53.55)
<i>Tamarindus indica</i>	50	63.3 (52.71)	44.6 (41.90)	100	67.3 (55.12)	54.4(47.35)
<i>Emblica officinalis</i>	50	64 (53.13)	46.4 (42.94)	100	69 (56.17)	63.8(54.21)
<i>Allium sativum</i>	50	81.3 (64.38)	70.4 (57.04)	100	90.6 (72.24)	81.2 (64.3)
<i>Terminalia bellirica</i>	50	84.6 (66.89)	73.7 (59.15)	100	95 (77.08)	88.2(69.91)
<i>Terminalia chebula</i>	50	75.3 (60.27)	67.1 (54.94)	100	84.3 (66.66)	76 (60.67)
<i>Mentha piperata</i>	50	70.3 (56.98)	54.4 (47.52)	100	70.6 (57.17)	61.5(51.65)
<i>Allium cepa</i>	50	72.3 (58.24)	60 (50.77)	100	85 (67.21)	68.5(55.86)
S.Em.		±2.55	±1.13		±2.70	±1.32
C.D. at 5%		-	-		-	-
C.D. at 1%		-	-		-	-

Diffusate of *T. chebula* showed an inhibitory effect against strain X-100 of the bacterium *Xanthomonas campestris* pv. *citri* indicating its usefulness for the management of citrus canker disease¹⁶. The plant extracts of *Allium cepa*, *Azadirachta indica*, *Tamarix aphylla*, *Vernonia anthelmentica*, *Plumbago zelanicum*, and *Tegetis erecta* showed significantly good antibacterial activity at 50% concentration against *X. campestris* pv. *campestris* *in vitro* and resulted in better seed germination and vigour than streptomycin¹⁷. Aqueous fruit extracts of *T. bellirica* and *A. sativum* individually were found most effective against the pathogen at 100% concentration while aqueous fruit extracts of *T. chebula* and leaf extracts of *M. piperita* showed good antibacterial efficacy at 50% conc. The inhibition annulus was in the range of 8.63 to 483.64 mm² (Table 1). The fruit extract of *T. chebula* showed maximum antibacterial efficacy (IA 483.64 mm²) followed by fruit extract *A. sativum* (IA 432.53 mm²). The relative sequence of inhibition annulus was as follows for the studied pathogen:

Terminalia bellirica > *Allium sativum* > *Allium cepa* > *Terminalia chebula* > *Emblica officinalis* > *Mentha piperata* > *Azadirachta indica* > *Tamarindus indica*

Seed treatment using standard blotter method

Aqueous plant extracts with significant inhibiting activity were used for *in vitro* seed treatment using standard blotter method. In the present study, aqueous extracts of *T. bellirica*, *A. sativum*, *A. cepa*, *T. chebula*, *E. officinalis*, *M. piperata*, *A. indica* and *T. indica* were used to treat seeds to observe percent seed germination and control of the pathogen. The seed treatment with these extracts also improved seed germination and control of pathogen. This may be due to the presence of bioactive compounds

in plant extracts¹⁸. Seed treatment with *T. bellirica* and *A. sativum* showed maximum seed germination and control of pathogen. Plant extracts of *T. chebula* is reported to be an antimicrobial, hepatoprotective, anti-inflammatory, immunomodulatory, an antioxidant and adaptogenic^{16,19,20,21}. Aqueous extracts of leaves of *Tamarindus indica* showed good antibacterial activity against Gram positive bacteria and hydroalcoholic extracts of leaves in Gram negative bacteria²². The fruit pulp extract of *T. indica* exhibited a wide spectrum of antibacterial activity against gram positive and gram negative bacterial strains²³. Garlic (*Allium sativum*) with its antimicrobial properties is widely used for number of infectious diseases. Hot water extract of garlic showed good inhibitory effect against plant pathogens, *Xanthomonas citri* and *Erwinia carotovora* and human pathogens²⁴. 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. bellirica > *A. sativum* > *A. cepa* > *T. chebula* > *A. indica* > *M. piperata* > *E. officinalis* > *T. indica*

A significant percent control of the pathogen was found at 100% conc. after treating seeds with *T. bellirica* (88.1%) and *A. sativum* (81.7%). After seed treatment with the plant extracts, seed germination also improved significantly. The relative percent control of the pathogen was as follows.

T. bellirica > *A. sativum* > *T. chebula* > *A. cepa* > *A. indica* > *E. officinalis* > *M. piperata* > *T. indica*



CONCLUSION

In conclusion our study revealed that among eighteen different plant parts used in study of aqueous plant extracts evaluated for their antibacterial activity, *Terminalia bellirica* (Fruit) and *Allium sativum* (Bulb) gave the promising results against *X. pisi* causing leaf spot disease of pea *in vitro* and also improved seed germination due to their antibacterial activity.

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