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Biological Control of Fruit Lesions Caused by *Xanthomonas campestris* Pathovars from *Cuscuta pedicellata* Ledeb. *In vitro*

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In search for alternative ways of bacterial fruit lesions control, we evaluated the aqueous decoction and aqueous infusion extract of *Cuscuta pedicellata* L. for their antibacterial activity against different pathovars of *Xanthomonas campestris* the causal agent of this disease. The collected leaves were washed, dried and powdered. Aqueous decoction and aqueous infusion extracts were prepared and observed their antibacterial efficacy by using the well diffusion method *in vitro*. The significant results were obtained show that the both extracts tested inhibited the bacterial growth of pathogen with inhibition zone diameter ranging from 1.0 to 5.0cm. Results evidenced the variation in susceptibility among test pathovars of *X. campestris* towards the *C. pedicellata* extracts. The aqueous decoction *of C. pedicellata* revealed strongest antibacterial activity against *X. campestris* pv. *punicae* and *X. campestris* pv. *mangiferaeindicae*. On the other hand, aqueous infusion extract exhibited effective antibacterial activity against *X. campestris* pv. *citri* and *X. campestris* pv. *pinicae*. The present investigation strongly indicates biological potential of *C. pedicellata* against different pathovars of Xanthomonads in the sustainable bacterial disease management in agriculture.

Key words: Antibacterial activity, *Cuscuta pedicellata*, Xanthomonads.

In general, synthetic pesticides are quick in management of various crop diseases and microbial contaminations in the filed of agriculture[1]. Studies have been proved that the continual and random applications of these pesticides are the main cause of biohazards and residual toxicity in the ecosystem[2]. In the present scenario, an environment friendly agent is needed to reduce the deleterious effects of chemical pesticides as well as for the sustainable management of pathogenic micro-organisms in field crops[3]. Biological control is an alternative to chemicals in the control of plant pathogens and to reduce environmental pollution. It has been well studied as non-hazardous approach in order to reduce crop damage caused by various phytopathogens[4]. *Xanthomonas campestris* is an important bacterial pathogen that can cause various diseases in a wide variety of crop plants and exhibits visible symptoms and shedding leaves. The disease results in reductions in market value and fruit yield[5]. Recently evaluation of plant extracts against many *Xanthomonas* species is becoming an important area. Thus a focus should also be given to indigenous practices of the farmer to look for their effectiveness. Especially the indigenous knowledge on plants is important. *C. pedicellata* belong to the family Convolvulaceae also known as Dodder. It is leafless green yellowish and thread like twinning herb. The stem shows antimicrobial activity against many fungi and bacteria[6]. Therefore, present study was carried out to

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evaluate antimicrobial potential of *C. pedicellata* extracts against different pathovars of *Xanthomonads* causing fruit lesions.

MATERIALS AND METHODS

Collection of plant materials

The fresh stems of *C. pedicellata* plant were collected from various places of University of the Punjab, Lahore, Pakistan. The stem samples were thoroughly washed in tap water and shade dried for three weeks. The dried samples were grinded to fine powder and preserved in air tight bottles at room temperature.

Preparation of aqueous infusion

Aqueous infusion of *C. pedicellata* was prepared by steeping 20 gm of powder in 60 ml sterile distilled water in sterile flasks. These flasks were incubated at room temperature with alternate shaking for 48 h. The contents of flasks were filtered[7].

Preparation of aqueous decoction

Aqueous decoction of *C. pedicellata* was prepared by boiling of 20 gm sample powder in 60 ml sterile distilled water up to 15 minutes. After cooling, the filtrate of aqueous contents was stored at 4°C for further use[7].

Isolation and Identification of pathogenic *X. campestris* species

X. campestris was isolated from different diseased fruit samples (Table 1) by serial dilution method. The infected fruits were surface sterilized with 70% ethanol and washed thoroughly with sterile water. A small infected piece was suspended and thoroughly mixed in 10ml of sterile water in sterilized test tube. A sterile pipette was used to transfer 1 ml of this suspension to a tube containing 9ml of sterile water. This process continued until the ten times of dilutions were made and inoculated on *Luria-Bertani* (LB) agar media plate by streak plating method and incubated at 37°C for 24 hours[8]. Identification of bacterial species was done by recording macroscopic and microscopic characteristics. The purified colonies were subjected to gram staining and characterized using biochemical tests and consulting the pertinent literature[8].

Antibacterial activity of *C. pedicellata* extracts

A modified well diffusion method was employed to evaluate the antibacterial activity of aqueous infusion and decoction extracts of *C. pedicellata*[9]. The standard bacterial suspension (McFarland 0.5) was made for each isolate of *X. campestris*. Eighty microlitres (80 µL) of bacterial inoculum of each isolate were evenly spread on the LB agar media surface with the help of glass spreader. The media surface were allowed to dry for 5 mintues and bore wells in the agar plates by a sterile cork borer of diameter 0.8 cm. Wells are filled with 60 µL volume of each aqueous infusion and decoction extracts and incubated at 37°C for 24 hours. Distilled water was used as negative controls in well. After incubation, inhibition zones around the well were measured and compared to the control. All tests were conducted in three repetitions arranged as one well in three plates for each extract[10]. Antibacterial activity Index was calculated as:

Activity Index (AI) = Da/ Db - 1

Where: Da is the diameter (cm) of the growth zone in the experimental dish and Db is the diameter of the growth zone in the control dish.

Statistical evaluation

The antibacterial activity of *C. pedicellata* was evaluated by diameter of zone of inhibition that is the mean of triplicates ± SE of three replicates.The treatment means were subjected to analysis of variance and were computed by Duncan's multiple range test (DMRT) at $P \leq 0.05$.

RESULTS

Morphological and Biochemical Characteristics of *X. campestris* species

Different pathovars of *Xanthomonads* were assessed in diseased samples of fruits. All samples were inoculated on sterilized plates of LB medium by serial dilution method and incubated at 37 °C for 24 hours. Identification was done by recording morphological and biochemical characters and consulting the pertinent literature of Bergey's Manual of Determinative Bacteriology (9th Edition)[8]. Pathovars of *Xanthomonads* showed mucoid growth with light to deep yellow, creamy (honey) color of colonies and were found to be gram negative. All pathovars tolerated 1% and 2% NaCl and gelatin liquefied within 3 to 21 days, able to reduce nitrate, produced H_2S gas, positive to indole production, oxidase reaction test negative

and catalase reaction positive to all. With regard to citrate and malate utilization did not utilize malate and citrate while utilized organic acids. In casein and starch hydrolysis ability, all pathovars were found positive for both casein and starch[8]. The antibacterial efficacy of aqueous decoction and aqueous infusion extracts of C. *pedicellata* were determined in vitro using the well diffusion method. Results obtained show that the two aqueous extracts inhibited different pathovar of X. *campestris* with inhibition zone diameter ranging from 1.0 to 5.0 cm (Table 2 and Fig. 1).

Inhibitory Effect of Aqueous Decoction Extract of *C. pedicellata* on Pathogen

The aqueous decoction *extract show evidence of high antibacterial activity against X. campestris* pv. *punicae* (5.0 cm) and *X. campestris* pv. *mangiferaeindicae* with 4.0 cm inhibition zone. *X. campestris* pv. *citri* has least resistance against this extract (2.1 cm) as compared to other pathovar species. In case of *X. campestris* pv. *musacearum* and *X. campestris* pv. *pruni* extract showed comparable behavior by being moderately active with 2.8 cm and 2.5 cm respectively. Although, *X. campestris* pv. *vesicatoria* exhibited intermediate inhibitory effect (3.0 cm) against aqueous decoction extract of *C. pedicellata*.

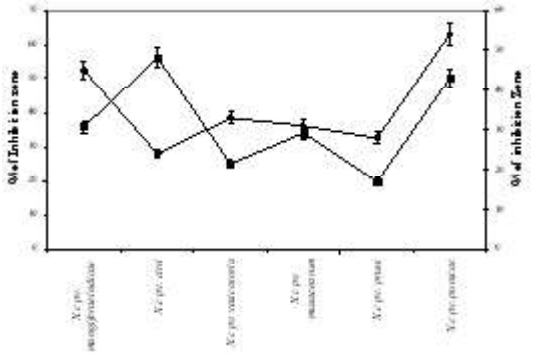

Fig. 1. Percentage of Inhibition Zone of Aqueous Decoction and Infusion extracts of *C. pedicellata* on different pathovar of *Xanthomonads* isolated from Diseased Fruits

Inhibitory Effect of Aqueous Infusion Extract of *C. pedicellata* on Pathogen

The aqueous infusion extract of *C. pedicellata* was significantly active against tested pathogenic isolates of *Xanthomonas* species. The extract showed high antibacterial activity (5.0cm) against *X. campestris* pv. *citri* followed by *X. campestris* pv. *mangiferaeindicae* and *X. campestris* pv. *musacearum* with inhibition zone of 3.2 cm and 3.1 cm respectively. The extract was also more active against *X. campestris* pv. *punicae* (4.5 cm) whereas the least activity was showed *X. campestris* pv. *pruni* (1.8 cm). The film growth of *X. campestris* pv. *vesicatoria* was moderately inhibited by the aqueous infusion extract of *C. pedicellata* with the zone of inhibition 2.2 cm.

DISCUSSION

Testing the antimicrobial activity of plants remains an area of intense interest. Many reports are available on the antiviral, antifungal, antibacterial, antihelmintic, antimolluscal, and anti-inflammatory properties of plant[11-15]. Although a range of serious environmental implications related with the excessive use of chemicals, until remains first line for defense against plant pathogen.

In vitro, antibacterial screening of *Cuscuta* sp., extracts has exhibited promising results that indicate its potential use in the management of *Xanthomonas* species in crop field. *Xanthomonas campestris* is a Gram-negative and rod-shaped bacterium that causes blights, cankers and leaf spots in various agricultural crops. *Xanthomonas* species are known to cause significant yield loss in agriculture[16]. Many applications of different antibiotics are used to

Table 1. Pathovars of *Xanthomonads* isolated from Diseased Fruits

Pathovar of *X. campestris*	Name of fruits	Source
X. campestris pv. *mangiferaeindicae*	*Mangiferae indica* (Mango)	Diseased Fruit, Lahore
X. campestris pv. *citri*	*Citrus sinensis* (Lemon)	Diseased Fruit, Lahore
X. campestris pv. *vesicatoria*	*Lycopersicon esculentum* (Tomato)	Diseased Fruit, Lahore
X. campestris pv. *musacearum*	*Musa acuminata* (Banana)	Diseased Fruit, Lahore
X. campestris pv. *pruni*	*Prunus persica* (Apricot)	Diseased Fruit, Lahore
X. campestris pv. *punicae*	*Punica granatum (Pomegranate)*	Diseased Fruit, Lahore

Table 2. Effect of *C. pedicellata* extract on the growth of different pathovar of *Xanthomonads* isolated from diseased fruits

Pathogens	Aqueous Decoction				Aqueous Infusion			
	Bacterial growth (control)	Inhibition zone (cm)	Experimental growth zone	Index	Bacterial growth (control)	Inhibition zone (cm)	Experimental growth zone	Index
X. campestris pv. *mangiferaeindicae*	9.0±0.0	4.0±0.06b	4.9±0.06b	0.43b	9.0±0.0	3.2±0.02c	5.8±0.03cd	0.34c
X.campestris pv. *citri*	9.0±0.0	2.1±0.01ef	6.8±0.03e	0.23f	9.0±0.0	5.0±0.03a	2.6±0.06a	0.70a
X. campestris pv. *vesicatoria*	9.0±0.0	3.0±0.03c	6.0±0.03c	0.33c	9.0±0.0	2.2±0.05e	6.7±0.03e	0.24d
X. campestris pv. *musaceearum*	9.0±0.0	2.8±0.03d	6.1±0.03cd	0.31cd	9.0±0.0	3.1±0.10cd	5.9±0.03c	0.34c
X. campestris pv. *pruni*	9.0±0.0	2.5±0.03de	6.5± 0.01d	0.28e	7.0±0.0	1.8±0.02f	7.1±0.10f	0.20de
X. campestris pv. *punicae*	9.0±0.0	5.0±0.03a	4.1±0.01a	0.54a	5.0±0.0	4.5±0.04b	4.5±0.03b	0.50b

Values with different letters show significant difference (P0.05) as determined by Duncan's Multiple Range Test.

control these diseases. Due to eco-friendly nature, bactericides chemicals from plants can be one alternative method in integrated disease management[17]. Furthermore, biopesticide are safe to end-user, the public and the radical environmentalists.

In this study, effects of antibacterial activity of *C. pedicellata* were tested against different pathovar of *X. campestris in vitro* condition. Both type of extract appear to be promising as a biocontrol agent against the pathogen. Previously, antimicrobial activity of *Cuscuta* species against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Penicillium citrium* and *Aspergillus niger* is also well summarized by Faiyyaz *et al*.[6]. Extract of stem of *Cuscuta* species showed the presence of alkaloids, carbohydrates, some glycosides, flavonoids, tannins, phenolic compounds, steroids and shows antimicrobial activity. The current results agree with findings of Faiyyaz *et al*.[6] that demonstrated the presence of antimicrobial activity in *C. pedicellata* extracts and have also reported the presence of above mentioned antimicrobial compounds.

CONCLUSION

Thus it can be concluded that the present experiment demonstrates that the stem extracts of *C. pedicellata* exhibit antibacterial effect which offers a scientific basis for using *C. pedicellata* as a good sources of antibacterial compounds. Further investigations are going on the efficacy in bio-control of other bacterial diseases in a variety of crops.

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