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Research Article

Antibacterial activity of a few medicinal plants against *Xanthomonas campestris* pv. *campestris*

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ABSTRACT

Antibacterial activity of 12.5%, 25% and 50% alcoholic extracts of leaves of twenty different medicinal plants were investigated by disc diffusion method against gram negative bacteria namely *Xanthomonas campestris pv. campestris* (plant pathogen) causing black rot disease on cauliflower. It was found that twenty extracts of all the plant samples showed significant activity against the tested bacteria. The plant extracts of *Allium cepa*, *Azadirachta indica*, *Tamarix aphylla, Vernonia anthelmentica, Plumbago zelanicum*, and *Tagetes erecta* showed significantly good antibacterial activity in 50% concentration against *X. c.* pv. *campestris in vitro* and resulted in better seed germination and plant vigour than streptomycin.

Keywords: Antibacterial activity; Black rot disease; *Brassica oleracea* (L.) var. *botrytis* L; Cauliflower; *Xanthomonas campestris pv. campestris*

INTRODUCTION

Xanthomonas is a very important kind of phytopathogenic bacteria, which causes the plant diseases all around the world. The hosts of this genus include at least 124 monocotyledonous and 268 dicotyledonous plants, among which the rice bacterial blight, cauliflower black rot disease, and citrus blight disease are the most serious diseases, which cause a big economic impact on agricultural production every year. Thus, pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crops (Mandavia et al., 1999).

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is the all time favorite vegetable and occupies an important place among the fresh vegetables in India and world. *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson is the causal agent of black rot of cauliflower, which is possibly the most important disease of crucifers worldwide (Williams, 1980). *X. campestris* pv. *campestris* is a small, rod shaped, aerobic gram-negative, non-spore forming bacterium (Onsando, 1992). Increasing public concern on environmental issues desires that alternative management systems be evolved either to reduce pesticide dependant or naturally occurring compounds be explored to constrain the pathogen attack (Cuthbertson and Murchie 2005; Singh 2003). Considering the deleterious effects of synthetic pesticides on life supporting system, there is an urgent need for alternative agents for the management of pathogenic microorganisms (Mahajan and Das, 2003).

In view of the economic impact of this plant pathogenic bacterium on vegetable production, the first and foremost objective of this study is to find out the strategies to curd the crop losses due to bacterial diseases. Therefore, the present attempt is one of such major steps in this direction as the studies on management of black rot of cauliflower through plant extracts.

MATERIALS & METHODS

Isolation and pathogenicity test of pathogen

For successful study black rot causing bacterium was isolated from naturally infected cauliflower plant leaves showing typical 'V shaped symptoms. The single fresh and pure glistering yellow droplets like colonies were purified on other petriplates having nutrient agar medium. Such culturally identical single colony was then transferred on yeast glucose chalk agar storage medium (Yeast extract - 3 g, Peptone - 5 g, Glucose - 5 g, CaCO3 - 20 g, Agar - 15 g, pH - 7.0) slants and maintained by preserving in a refrigerator for all future experiments, and making periodic transfers after every fortnight. Pathogenicity of the bacterium was proved on cauliflower plants by following Koch's Postulate on one-month-old plants, raised in 25 cm earthen pots in green house by using 48 hrs old bacterial growth cultures. Inoculation was done by carborundum abrasion method. The stock culture was kept in refrigerator after covering culture slants with sterilized mineral oil.

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Table 1: Medicinal plant species selected for antibacterial activity

Plant species	Local name	Family	Part used	Traditional uses
Acmella oleracea	Akrarkara	Asteraceae	Leaves	A decoction or infusion of the leaves and flowers is a traditional remedy for stammering, toothache and throat complaints.
Euphorbia tisucalla	Badidudhi	Euphorbiaceae	Whole plant	It has also been used as an application for asthma, cough, earache, neuralgia, rheumatism, toothache, and warts in India.
Aegle marmelos	Beal	Rutaceae	Leaves	A decoction of the leaves is a febrifuge and expectorant and is particularly used for asthmatic complaints. Also used to treat acute bronchitis, fever and dysentery.
Plumbago zelanicum	Chitrak	Plumbaginaceae	Flowers, Roots	Flowers promote appetite, helps in treating indigestion, dyspepsia, piles and skin diseases. The powdered root is taken as a snuff to relieve headache.
Datura stramonium	Datura	Solanaceae	Whole plant	It is used as an herbal medicine to relieve asthma symptoms and as an analgesic. It is a powerful hallucinogen and detriant.
Tamarindus indica L.	Imli	Fabaceae	Leaves	It is used in traditional medicines and metal polishes, used in deserts. It has an antiseptic property and for scurvy and even cough cure.
Tamarix aphylla (L.) Karst	Frash	Tamaricaceae	Whole plant	It is used as a barrier to fire considered weed for national significance They are used as windbreaks and shade trees.
Allium cepa L.	Onion	Amaryllidaceae	Shoots, Bulb	It is used in various dishes and salads. .Its tissue is used in science education. It has anti-inflammatory, anticholestrol, anticancer and antioxidant properties.
Blumea balsamifera L. DC	Sambong	Asteraceae	Leaves	The leaves are known for its ngai or Blumea camphor that is used as herbal medicine to treat kidney stones, wounds and cuts, rheumatism, anti-diarrhea, anti spasms, colds and coughs and hypertension.
Caesalpinia Bonducella L.	Karnju	*Caesalpiniaceae*	Seeds	In India seeds are mixed with black pepper to make a tonic and to reduce fevers. A tonic is also made from the bark.
Lantana camara L.	Lantana	Verbenaceae	Whole plant	Leaves show healing of gastric ulcers. Extracts of leaves are antibacterial, antipyretic, carminative and in treatment of respiratory system infections.
Tagetes erecta	Marigold	Asteraceae	Flowers	It is used as skin wash and for yellow dye. They can protect certain crop plants from nematode pests when planted in fields. It can be shown to kill gram negative and gram positive bacteria, oil of flower may be added to perfumes and contains antioxidants.
Muraya Koenigii	Meetha neem	Rutaceae	Leaves	The leaves are anti-diabetic, antioxidant, antimicrobial, anti inflammatory, hepatoprotective and anti hypercholesterolemic. It also contains iron.
Glycyrrhiza glabra L.	Mulatti	Fabaceae	Roots	It has anti inflammatory, antiviral, saponin, glycyrrhiza and many important chemicals that are required for good health.

Plant species	Local name	Family	Part used	Traditional uses
Azadirachta indica A. (Juss)	Neem	Meliaceae	Leaves	Non-drying oil is extracted from the seeds. It is used for soapmaking and to treat skin diseases, locally. The bark and leaf extracts are used as a tonic, and to reduce fevers.
Catharanthus roseus L.	Sadabahar	Apocynaceae	Whole plant	Diabetes, swelling of body , menorrhoa
Moringa olcifora	Sanjana	Moringaceae	Whole plant	It improves nutrition, boost food security, foster rural development, and support sustainable land-care. It may be used as forage for livestock, a micronutrient liquid, a natural anthelmintic and possible adjuvant
Rawolfia serpentina	Sarpgandha	Apocynaceae	Whole plant	It has been used for relief of various central nervous system disorders. Extracts of the roots are used for the intestinal disorders particularly diarrhea and dysentery and anthelmintic.
Ocimum basilicum	Tulsi	Lamiaceae	Whole plant	It is considered to be antibacterial, antifungal, antispasmodic, carminative, diaphoretic, digestive, emmenagogue, expectorant, stimulant, stomachic, refrigerant etc. The plant is generally used in treatments of problems concerning digestion and nervous system
Vernonia anthelmentica Wild.	Vernonia	Asteraceae	Whole plant	Plant pacifies vitiated vata, kapha, roundworm and threadworm infestation, cough, urinary retention, inflammation, skin diseases, pruritus, abdominal colic, fever and leucoderma.

Collection of plant materials and Preparation of Extracts

Fresh plants materials *viz.* leaves, bulbs and roots were collected randomly from Indian Agricultural Research Institute campus, Pusa, New Delhi. The plants together with their medicinal uses and common names are given in Table 1.

The extracts of 20 different plant leaves, bulbs and rhizomes were used to examine the inhibitory effects on bacterial growth. The plant samples were first washed, surface sterilized (2% sodium hypochloride) and kept in sterilized covered beaker and air-dried. All plant materials were weighed, crushed with sterilized distilled water (1:1 W/V) and 80% ethanol, and homogenized for five minutes in mortar and pestle. The mixture was filtered through two folds of muslin cloth and the filtrate was centrifuged at 5000 rpm for 15 min. The ethanol was evaporated and clear supernatant was diluted with distilled water to make a volume of 1:1 W/V. This was considered as 100 per cent concentration, which was used for the experiment (Singha and Saxena, 1989).

***In vitro* efficacy of plant extracts**

One ml of bacterial suspension, prepared in sterilized distilled water by 48 hrs old bacterial culture was incorporated in 250 ml of bioassay medium (peptone10g, beef extracts 3g, yeast extracts 5g, agar 20g.) separately, mixed thoroughly and 30 ml of medium by equal volume was poured in sterilized petriplates. The effectiveness of these plant extracts was tested by disc diffusion technique at 12.5, 25 and 50 % concentration. The petriplates were then incubated at 10^0 C for 15 min for allowing the chemical from disc to diffuse in the medium and later incubated at ambient temperature (28 ± 2^0 C) in BOD incubator with streptomycin (300ppm) chemical and untreated control. The inhibition zone was examined and per cent inhibition of growth was recorded after 72 hrs of incubation by using the following formula (Bliss, 1934). I = C-T/Cx100. Where, I = Inhibition per cent, C = Colony diameter in control (mm), T = Colony diameter in treatment (mm)

Effect of seed treatment on seed germination and plant vigour index

Mentioned 20 botanicals were tested to examine the effects on seed germination and seedling vigour of seeds inoculated with pathogenic strain *X. c.* pv. *campestris*.

The seeds of cauliflower were inoculated for 6 hrs with *X. c.* pv. *campestris* bacterial pathogen suspension and thereafter, seeds were treated with botanicals (by one hr seed soaking). Treated seeds were plated on three layers of moistened sterilized blotter paper kept in sterilized petriplates @ 10-20 seeds per petriplate and incubated at 28 ± 2^0 C. Sterilized distilled water was used to maintain the humidity in petriplates. The seeds treated with streptomycin (300 ppm) and untreated seeds served as control. Observation was recorded on per cent seed germination and seedling vigour (epi-

Table 2: *In vitro* efficacy of different medicinal plant extracts against *Xanthomonas campestris* pv. *campestris*

Plant extracts	Inhibition zone (mm) at different concentration (%)*			Mean
	12.5	25	50	
Spilangas ekmela	0.00	0.00	0.00	0.00
Euphorbia tisucalla	0.00	0.00	0.00	0.00
Aegle marmelos	5.25	11.35	15.42	10.67
Plumbago zelanicum	7.35	15.42	20.42	14.39
Datura stramonium	6.31	13.45	19.25	13.00
Tamarindus indica L.	0.00	3.51	6.15	3.22
Tamarix aphylla (L.) Karst	8.45	16.26	24.45	16.38
Allium cepa	9.45	18.62	24.87	17.64
Glumea aromatica	2.75	5.89	8.62	5.75
Cesellpinia badallelia	4.31	9.28	14.35	9.31
Lantana camara L.	5.62	10.95	15.95	10.84
Tagetes erecta	6.34	14.25	20.46	13.68
Muraya canicai	4.25	8.61	14.69	9.18
Glycyrrhiza glabra L.	5.01	10.27	15.65	10.31
Azadirachta indica A. (Juss)	7.88	17.96	23.75	16.53
Vinaca rosea	1.78	3.62	5.87	3.75
Moringa olcifora	5.61	11.61	15.26	10.82
Rawolfia serpentina	2.81	4.72	8.35	5.29
Ocimum basilicum)	5.16	9.87	14.35	9.79
Vernonia anthelmentica Willd.	6.98	15.58	22.83	15.13
Streptomycin (300 ppm)	24.42	24.42	24.42	24.42
Control	0.00	0.00	0.00	0.00
CD (*P*=0.05) Treatment 0.352,Concentration 0.706 , Treatment x Concentration 0.972				

***** Average of three replications

cotyl and hypocotyl length) after 10 days of inoculation and plant vigour index was calculated by following formula described by Abdul Baki and Anderson (1973). Plant Vigour Index = (Mean epicotyl length + Mean hypocotyl length) x Germination per cent. The experiment was repeated thrice.

RESULTS AND DISCUSSION

In vitro efficacy of plant extracts

A perusal of data documented in Table 2 elucidate that the maximum inhibition zone (24.87 and 24.45 mm) was obtained from *Allium cepa* and *Tamarix aphylla* extracts at 50 per cent dilution, which is higher than streptomycin 300 ppm (24.42 mm) against the black rot bacterium. However, *Azadirachta indica*, *Vernonia anthelmentica*, *Tagetes erecta* and *Plumbago zelanicum* also showed very good inhibition zone 23.75, 22.83, 20.46 and 20.42 mm, at its 50% concentration, respectively. Nonetheless, *Datura stramonium*, *Moringa olcifora*, *Glycyrrhiza glabra*, *Lantana camara* and *Aegle marmelos* extract were fairly good in inhibiting all the strains.

Effect of seed treatment on seed germination and plant vigour index

Among twenty various plant extracts the *A. indica*, *A. cepa*, *P. zelanicum*, *D. stramonium* and *V. anthelmentica* extracts enhanced much more seed germination (> 85%), fresh and dry weight of seedlings as compared to inoculated control and other treatments. However, the streptomycin was significantly higher than other plant extracts in seed germination (94.6%). The seedling vigour was found less than that of uninoculated control except in *A. cepa* (1008.7), *A. indica*(1026.2) and streptomycin (1014.1). The highest dry weight mg per plant was observed with the treatment of streptomycin (144 mg) *A. cepa* (116mg) followed by *A. indica* (114 mg), *P. zelanicum* (112 mg), and *V. anthelmentica* (110 mg) over other plant extracts and uninoculated control treatments (105 mg).

Lirio et al. (1998) has also reported antibacterial activity of *Allium cepa, A. porrum, A. sativum, Euphorbia tisucalla* and piper bettle against *Erwinia carotovora* pv. *carotovora*, *X.c.* pv. *campestris* and *Pseudomonas solanacearum* when used in aqueous extracts. In a similar study, the leaf extracts of *Catharanthus roseus* was found to have significant antibacterial activity against *Xanthomonas campestris* (Satish et al., 1999) .Sharma and Mehta (2001) reported that leaf extracts from *Prosopis juliflora, Allium sativum, Vitis quadranquralis, Carucuma longa, Occimum sanctum* and *Eucalyptis citridosa* contained antimicrobial activity against *X. c.* pv. *campestris.* The antibacterial potential of *Melia azedarach* L. was tested using crude leaves, flower and fruit-seed extracts against pathogenic bacterial strains (Abdul vigar et al., 2008) Pawar and Papdiwal (2010)

Table 3: Effect of plant extracts and *Xanthomonas campestris pv. campestris* on germination percentage and growth parameters of cauliflower seedling

Plant extracts	Percent Germination (%)	Average shoot length (cm)	Average root length (cm)	Average fresh weight (g)	Average dry weight (mg)	Vigour index
Spilangas ekmela	77.8 (61.21)	5.25	4.45	1.84	85	754.6
Euphorbia tisucalla	76.2 (60.80)	4.81	4.32	1.54	74	695.7
Aegle marmelos	76.5 (61.00)	5.31	4.34	1.76	78	738.2
Plumbago zelanicum	87.3 (69.12)	5.35	5.25	2.10	112	925.3
Datura stramonium	86.4 (68.36)	5.34	5.28	2.05	108	917.5
Tamarindus indica L.	78.5 (62.38)	3.32	2.45	1.90	97	452.9
Tamarix aphylla (L.) Karst	83.4 (65.96)	4.32	3.25	2.05	105	631.3
Allium cepa	93.4 (75.11)	5.45	5.35	2.15	116	1008.7
Glumea aromatica	76.8 (61.21)	5.32	4.38	1.74	77	744.9
Cesellpinia badallelia	75.3 (60.20)	4.32	3.15	1.52	73	562.4
Lantana camara L.	78.2 (62.17)	4.25	3.31	1.92	98	591.2
Tagetes erecta	80.3 (63.65)	5.31	5.25	2.00	102	847.9
Muraya canicai	77.9 (61.96)	5.25	4.75	1.86	92	779.0
Glycyrrhiza glabra L.	77.4 (61.61)	5.18	4.73	1.82	85	767.1
Azadirachta indica A. (Juss)	94.5 (76.44)	5.44	5.42	2.12	114	1026.2
Vinaca rosea	76.7 (61.14)	5.23	4.62	1.75	79	755.5
Moringa olcifora	77.4 (61.61)	5.34	4.75	1.83	84	780.9
Rawolfia serpentina	75.8 (60.53)	3.24	2.35	1.49	79	423.7
Ocimum basilicum)	75.3 (60.20)	4.92	3.53	1.52	73	636.3
Vernonia anthelmentica Willd.	85.6 (67.70)	5.35	5.25	2.09	110	907.4
Streptomycin (300 ppm)	94.6 (76.56)	5.30	5.42	1.85	144	1014.1
Uninoculated control	90.3 (71.85)	5.43	5.45	1.80	105	982.5
Inoculated control	34.5 (35.97)	2.45	3.36	1.46	63	200.5
CD (*P*=0.05)	3.369	0.255	0.214	0.083	2.671	35.629

* Values in parentheses are arcsine-transformed values

screened fresh leaf extracts of 30 plants against 11 strains of *Xanthomonas campestris* pv. *mangiferaeindicae* (*Xcmi*) out of which, 12 leaf extracts showed antibacterial activity. The extract of *Terminalia thorelii* and *Azadirachta indica* showed maximum activity against the *Xcmi* strains under investigation. Sheikh et al. (2012) studied the antimicrobial potential of eleven different aqueous leaf extracts on *Xanthomonas campestris*, *Agrobacterium rhizogenes* and *Aspergillus fumigatus* based on formation of the zone of inhibition (ZOI). *Prosopis juliflora* showed maximum and significant inhibitory effect on the growth of all the three pathogens. In case of *Xanthomonas campestris* the effect of this plant extract was almost equal to the strength of Streptomycin 10 mcg, (the metric system uses the term mcg to represent micrograms). Neycee et al. (2012) evaluated the antibacterial effects of methanol extract of seed-fruits, leaves and flowers of chinaberry (*M. azedarach*) via growth inhibitory zone assay using disc diffusion experiments against strains of *Pseudomonas syringae pv. syringae*, *Xanthomonas campestris pv. campestris*, *Rathayibacter tritici* and *Escherichia coli*. Results showed positive effect and size of growth inhibition zone in each of the factors A, B, C (organ, concentration and bacteria) showed a significant difference, while there was also a significant difference between types of organ and bacteria in forming growth inhibitory zone.

CONCLUSION

Comparatively the present investigation showed that bacterial growth inhibition is not only single factor prior to recommendation in the field condition for disease control. There should be the thorough tests for seed germination and plant vigour index for a successful disease management. The use of plant extracts viz. of *Allium cepa*, *Azadirachta indica*, *Tamarix aphylla, Vernonia anthelmentica, Plumbago zelanicum*, and *Tegetis erecta* in combination with streptomycin can be a part of integrated disease management of black rot of cauliflower.

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