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Potential use of some plant extracts as bio-protectant against bacterial canker of peach

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ABSTRACT

The antibacterial activity of twenty plants samples was assessed against Pseudomonas syringae pv. syringae, a causal organism of bacterial canker of peach. The combined extracts of seed of Dedonia viscosa and petal extracts of Lantana camera showed a strong enhancement in antibacterial action over the individual extracts. Other plants such as Acacia arabicae, Lantana macrophylae and Mimosa hamata also showed the inhibitory effect against the test bacteria.

INTRODUCTION

The bacterial canker, caused by *Pseudomonas syringae* pv. *syringae*, van Hall, has been known to occur in stone fruits since late 1800 and is responsible for causing diseases in cultivated cherry, plum, peach, and apricot as well as wild cherry all over the world and, causes significant economic losses [6]. Bacterial canker of plum caused by *P. syringae* pv. *syringae* causes annual tree mortality rates as high as 30% in Germany [5]. Many attempts have been made by different workers to manage the diseases by treating with chemical compounds [13] that are ultimately added to the natural environment and result in serious health issues. To avoid the use of these synthetic chemicals, the plants and their product may be utilized to combat phytopathogens as plants are known to possess various secondary metabolites having antibacterial properties [10-13]. Efficacy of some plant extracts to control bacterial canker disease of peach was assessed in this study.

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MATERIALS AND METHODS

The various parts of twenty plant were collected from different region of Haryana and its neighboring states on the basis of their traditional values [4] as shown in Table 1. The collected plant materials were thoroughly washed with tap water, followed by distilled water and then kept in dark on filter papers at room temperature till completely dried. Each sample was individually grounded into powder form for preparation of extract. The bacteria *Pseudomonas syringae pv. syringae* used for the study was procured from the cultures stocks of our Department. The stock culture was maintained at 4ºC on Nutrient Agar medium with periodic sub-culturing.

Antibacterial tests

Fifteen percent extracts of various plant/parts were prepared (15g/100ml water) by brewing in boiling water for 15 minutes followed by centrifugation at 12000 rpm for 15 minutes. The extracts were autoclaved for 15 minutes at 121ºC and the pH was adjusted to 7.0 with 2.5 mol/ 1 NaOH or 2.7 mol/ 1 HCl [11]. The antibacterial

Table 1: Common names, families and traditional uses of plants used in the present study

Sr. No.	*Botanical Name*	*Common Name*	*Name of Family*	*Distribution*	*Traditional Uses of Plants*
1.	*Acacia arabicae* Willd.	Kikar	*Mimosaceae*	India and Tropical Africa	Used for making furniture's, tanning, dyeing fabrics yellow, stem yields gum while seeds are fermented with dates to give beverages [12].
2.	*Anthocephalus cadamba* (Mig.)	Kadam	*Rubiaceae*	Tropical Asia	The bark is used as a tonic and reduces fever [12].
3.	*Cassia nodusa* (Ham.)	Gulabi Amaltash	*Caesal-piniaceae*	West Malaysia	The wood is used for posts and tool handles while roots are used as soap for washing clothes [12].
4.	*Dedonia viscosa* (L.)	Ailyer	*Sapindaceae*	Tropics & Subtropics, Australia	Fruits used as hops in manufacture of beer, bark as an astringent and a poultice [12].
5.	*Jacranda mimosaefolia* (D.Don.)	Nili Gulmohar	*Bignoniaceae*	Tropical South America	The wood is used in general carpentry [12].
6.	*Lagerstroemia flos-reginae* (Retz.)	Jarul	*Lythraceae*	Malaysia	The wood is insect resistant and used for house building, flooring, bridges and railways sleepers [12].
7.	*Lantana camera (L.)*	Ghaneri	*Verbenaceae*	Tropical America	A decoction of the leaves is used locally as a tonic and stimulant [12].
8.	*Lantana macrophylae(Mart.)*	Ghaneri	*Verbenaceae*	South America	A decoction of leaves is used in Brazil to treat rheumatism and the fruits are used to make a tonic [12].
9.	*Lawsonia alba (L.)*	Mahendi	*Lythraceae*	Old World Tropics, N. Africa, Arabia to India.	The bark used to treat jaundice and nervous complaints, flowers yield a scented oil, dried leaves yield a green powder used to dye hair, palm and nails orange brown (Henna) and to dye horses coats and fabric [12].
10.	*Melia azadirachta (L.)*	Neem	*Meliaceae*	East India, Ceylon	Non-drying oil is extracted from the seeds. It is used for soap-making and to treat skin diseases, locally. The bark and leaf extracts are used as a tonic, and to reduce fevers [12].
11.	*Mimosa hamata(Willd.)*	Aill	*Mimosaceae*	Tropical Asia	Tonic, in urinary complaints, glandular swellings, blood-purifier [12].
12.	*Murraya koenigii(Kurz.)*	Kadi Pata	*Rutaceae*	East Asia, , Pacific Islands, Himalayas.	A decoction of the bark leaves and root is used locally as a tonic [12].
13.	*Musa paradisiaca (L.)*	Kela	*Musaceae*	Tropical Asia	The high starch content of the fruits, flour from the fruit is an excellent invalid food [12].
14.	*Nerium indicum (Mill.)*	Red Kaner	*Apocynaceae*	Tropical Asia	A poultice of the root is used against ringworm, to induce abortion and for suicide; flowers are used for perfume and produce good honey [12].
15.	*Nicotiana tabocum (L.)*	Tamakhu	*Solanaceae*	Tropical America	The cured and dried leaves are used to make tobacco, snuff ans a source of nicotine for the manufacture of insecticides and nicotine sulphate [12].
16.	*Nychtenthus arbor-tristis (L.)*	Har Sringar	*Verbenaceae*	India	The leaves yield a bright yellow dye [12].
17.	*Ocimum basilicum (L.)*	Ban Tulsi	*Labiatae*	India, S.E. Asia, N. E. Africa	The plant is cultivated for the essential oil used in perfumery, soap making, to flavour liqueurs and sauces [12].
18.	*Ocimum sanctum (L.)*	Tulsi	*Labiatae*	Old World Tropics	The plant is sacred to the Hindus and is grown in front of temples; the leaves are used as a condiment [12].
19.	*Onosoma echinoids (L.)*	Inderjo	*Boraginaceae*	Central Europe To Himalayas	The roots yield a red dye (Orsanette) used in India to dye fats and wool, in place of Alkanna [12].
20.	*Phoenix dactylifera*	Khajur	*Palmae*	California, N. America, Minor Asia	Fruits are eaten fresh or dried, mixed with milk or fermented to make alcoholic beverages [12].

activity was tested by agar diffusion method with slight modification [11]. Bacterial suspensions were cultured in peptone water for 6-8h and 0.2ml of this culture was spread on Mueller – Hinton agar in Petri dishes. Wells (8mm diameter in size) were cut in agar plates and were filled 0.1ml of 15% plants extracts. The plates inoculated with *Pseudomonas syringae pv. syringae* were incubated at 37º ± 2ºC. The resulting zone of inhibition was measured after 24 h. Each combination of isolates and antimicrobial agent was repeated three times. The isolate which showed clear zone of inhibition more than 12mm including the 8mm well size were considered sensitive and those with less than 12mm as resistant.

Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method [6] where plants samples concentration ranged from 0.25% – 3.0% and defined as the lowest concentration that prevented visible growth of microorganisms after incubation for 40hours at 37° ± 2°C.

Assay for antibacterial activity of combined plant samples

The sample of each plant was prepared as explained earlier. The selected plants extracts were combined in the ratio 1:1. Assay for the antibacterial activity was done by agar diffusion method with slight modification [11].

RESULTS

The activity of the plants extracts against the bacterial growth of *Pseudomonas syringae pv. syringae* is presented in Table 2. It was observed that out of 20 plants extracts tested, the petals extracts of *Lantana camera* (30.0 mm) showed marvelous inhibitory effect against the bacterial growth of *Pseudomonas syringae pv. syringae*. The appreciable inhibitory effect was also shown by seeds extracts of *Dedonia viscosa* (19.5 mm), leaves extracts of *Acacia arabicae* (18.5 mm) and seeds extracts of *Mimosa hamata* (16.0 mm) against the test bacteria. The test bacterium was

Table 2: Anti-bacterial activity and minimum inhibitory concentrations (MIC) of plant extracts against *Pseudomonas syringae pv. syringae*

Sr. No.	Name of Plant	Part Used	Zone of Inhibition (mm)*	Minimum Inhibitory Concentrations (%)				
				0.25	0.5	1.0	2.0	3.0
1.	*Acacia arabicae* (Willd.)	Leaf	18.5 ± 0.85	+	+	-	-	-
2.	*Anthocephalus cadamba* (Mig.)	Stem	---	NT	NT	NT	NT	NT
3.	*Cassia nodusa* (Ham.)	Seed	---	NT	NT	NT	NT	NT
4.	*Dedonia viscosa* (L.)	Seed	19.5 ± 0.92	+	+	+	-	-
5.	*Jacranda mimosaefolia* (D.Don.)	Seed	---	NT	NT	NT	NT	NT
6.	*Lagerstroemia flos-reginae* (Retz.)	Seed	---	NT	NT	NT	NT	NT
7.	*Lantana camera* (L.)	Petal	30.0 ± 0.60	+	+	+	-	-
8.	*Lantana macrophylae* (Mart.)	Leaf	13.5 ± 2.64	+	+	+	-	-
9.	*Lawsonia alba* (L.)	Leaf	11.5 ± 2.38	+	-	-	-	-
10.	*Melia azadirachta* (L.)	Seed	---	NT	NT	NT	NT	NT
11.	*Mimosa hamata* (Willd.)	Seed	16.0 ± 1.95	+	+	+	-	-
12.	*Murraya koenigii* (Kurz.)	Leaf	---	NT	NT	NT	NT	NT
13.	*Musa paradisiaca* (L.)	Leaf	---	NT	NT	NT	NT	NT
14.	*Nerium indicum* (Mill.)	Leaf	---	NT	NT	NT	NT	NT
15.	*Nicotiana tabocum* (L.)	Leaf	---	NT	NT	NT	NT	NT
16.	*Nychtenthus arbor-tristis* (L.)	Leaf	---	NT	NT	NT	NT	NT
17.	*Ocimum basilicum* (L.)	Leaf	---	NT	NT	NT	NT	NT
18.	*Ocimum sanctum* (L.)	Leaf	---	NT	NT	NT	NT	NT
19.	*Onosoma echinoids* (L.)	Pod	---	NT	NT	NT	NT	NT
20.	*Phoenix dactylifera*	Stem	---	NT	NT	NT	NT	NT
Lantana camera (Petal) *Dedonia viscosa* (Seed)		Combine Extracts	32.0 ± 0.46	+	-	-	-	-

*Mean ± SD NT = Not Tested

less inhibited by leaf extract of *Lantana macrophylae* (13.5 mm) and leaf extract of *Lawsonia alba* (11.5 mm). The thirteen plants samples did not show any antibacterial effect against the test bacteria. The combined extracts of *Lantana camera* petals and *Dedonia viscosa* seeds showed an enhancement in activities (32.0 mm) over the individual extracts (Table- 2). The MIC of two plants samples i.e. *Lantana macrophylae* and *Lawsonia alba* were 2.0% for the test bacteria followed by 1.0% *Acacia arabicae*, *Dedonia viscosa* and *Mimosa hamata,* while, *Lantana camera* including combined extracts of *Lantana camera* and *Dedonia viscosa* registered a MIC of 0.5% for *Pseudomonas syringae pv. syringae*.

DISCUSSION

Considering the need for an alternative eco-friendly approach to control the phyto pathogens, it is worthwhile to screen the antibacterial efficacy of locally available flora. The results obtained in our study clearly testify the potential of certain plant extracts against. *Pseudomonas syringae pv. syringae* and a definite potential for developing new effective bactericides. Among different plants, the seeds extracts of *Lantana camera* showed strongest inhibitory activity against *P. syringae pv. syringae*, which could be due to the presence of some antimicrobial phytochemicals [3, 10, 12]. Hence, spraying extracts of *Lantana camera* could be used for protecting plants against this bacteria. The seeds extracts of *Dedonia viscosa* also showed inhibitory activity against *Pseudomonas syringae pv. syringae*, due to antimicrobial phytomolecules present in them [2, 12]. The leaves extracts of *Acacia arabicae* and seeds extracts of *Mimosa hamata* also showed similar inhibitory effect against the bacterial growth of test bacteria.

The combined extracts of leaves of *Lantana camera* + seeds of *Dedonia viscosa* showed a synergistic enhancement in activities over their individual extracts. Possible reasons for this synergy may be due to: (a) Greater concentration of the various groups of botano-chemicals than in case of individual extracts due to additive effect of the extracts; (b) Greater diversity of the various groups of botano-chemicals due to supplementation by one or the other plant extracts; (c) The possibility of synergistic effect of the botano-chemicals in the cocktail. Therefore, the spray of the combined extracts of leaves extracts of *Lantana camera* + seeds extracts of *Dedonia viscosa* can be strongly recommended for protecting bacterial canker of peach crops caused by *Pseudomonas syringae pv. syringae* and a strong substitute of synthetic chemicals.

CONCLUSION

The study has shown that some plants namely *Lantana camera*, *Dedonia viscosa*, *Acacia arabicae* and *Mimosa hamata* are very effective in inhibiting the bacterial growth of *Pseudomonas syringae pv. syringae*. These plants could be further subjected to field trials to access their effectiveness in field conditions and can subsequently be explored for the possibilities towards the identification of the key bioactive agents, through implying modern Microbiology and Biochemical techniques.

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