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Potential Use of some Petal Extracts against *Xanthomonas campestris* pv. *campestris*

S.K. Bhardwaj[1*], S.K. Singla[2] and R.K. Bhardwaj[3]

[1]*Horticulture Wing, M.D. University, Rohtak- 124 001, India*
[2]*Divisional Forest Office, Ponta Sahib- 173025, India*
[3]*Department of Chemistry, Dyal Singh College, Karnal- 132001, India*
[*]*E-mail*: *skbmdu66@gmail.com*

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Abstract
The aqueous petal-extracts of 20 plants were screened by agar diffusion methods for their antibacterial activity against *Xanthomonas campestris* pv. *campestris*, a causal organism of black rot of cabbage and cauliflower. *X. campestris* pv. *campestris* was found most sensitive to the petal extracts of *Tagetes erecta* and *Chrysanthemum coronarium*. Some of the other plants such as *Acacia fernesiana, Anthocephalus cadamba*, *Bombax malabaricum*, *Lathyrus odoratus*, *Rosa damascena* and *Thevetia nerifolia* also showed the inhibitory effect against the test bacteria.

Key words: *Xanthomonas campestris* pv. *campestris*, antibacterial activity, petal extracts, phytochemicals.

Introduction

Nature is the foremost physician. Abuse of nature's law upsets the ecosystem leading to occurrence of diseases in plants. Plants, like humans and animals, also get sick, exhibit disease symptoms, and die. Plant diseases are caused by environmental stress, genetic or physiological disorders and infectious agents including viriods, viruses, bacteria, fungi, algae, insects and nematodes etc. Plant diseases of economic crops alone cause 13-20% annual loss on production representing US \$$50\times10^9$ (James, 1981). In the absence of exact estimates in India, it can safely be assumed that more than 50% of the others crops loss is due to soil inhabiting microorganisms. Now a days more and more hazardous chemicals are being added in the natural environment in order to maintain the productivity of crops, which enter the food chain through water, soil and air resulting serious harmful affect in human health (Ramachandra and Nagarathna, 2003). According to the survey made by the WHO, more than 50,000 people in developing countries are annually poisoned and 5,000 die as a result of the effects of toxic agents, used in agriculture. In India, 35,000-40,000 tons of hazardous chemicals are sprayed on the crops every year, instead of helping the poor, these chemicals are causing cancer, sterility and death (Das, 1983). To avoid the use of these horrible diseases causing synthetic chemicals, the plants and their product should be utilized to combat phytopathogens. Plants are known to possess various secondary metabolites having antibacterial properties against phytopathogens (Patni *et al*., 2005; Bhardwaj and Laura, 2008). Therefore, the efforts are underway to search economic and safe phytochemicals, which could be utilized for disease control. Thus the object of present study was to exploit the potential of different petals extracts against *X. campestris* pv. *campestris* for selecting the most potent plants possessing antibacterial activities.

Materials and methods

The petals of 20 plants mentioned in table 1 were collected from the nursery and horticulture wings. The samples were thoroughly washed with distilled water, dried in dark room and grounded into powder for extract preparation (15 g petals /100 ml water). The bacteria *X. campestris* pv. *campestris* (MTCC No. 2286) used for the study was procured from the IMMTC, Chandigarh. The culture was maintained at 4°C on nutrient agar medium with periodic sub-culturing.

Antibacterial tests

Plants extracts was prepared by brewing in boiling water for 15 minutes followed by centrifugation at 12000 rpm for 15 min. The supernatants were collected in screw-capped vials and sterilized by autoclaving for 15 min at 121ºC and the pH was adjusted to 7.0 (Toda *et al*., 1989).

The assay for antibacterial activity of each plant part extract was tested by agar diffusion method by slightly modifying the methods of Toda *et al*. (1989). Bacterial suspensions were cultured in peptone water for 6-8 h and 0.2 ml of this culture was spread on Mueller-Hinton agar in petridishes. Wells (8 mm diameter) were cut in agar plates and were filled 0.1 ml of 15% plants extracts. The plates inoculated with *X. campestris* pv. *campestris* were incubated at 30±1ºC. 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 12 mm including the 8 mm well size were considered sensitive and those with less than 12 mm as resistant.

Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method (Koneman *et al*., 1988) where plants extract concentration ranged from 0.25-3.0%. MIC was determined as the lowest concentration that prevented visible growth of microorganisms after incubation for 40 h at 30±1ºC.

Results

The activity of the plant-extracts against the bacterial growth of *X. campestris* pv. *campestris* is presented in table 2. It was observed that out of 20 plants parts extracts tested, nine plant extracts showed inhibitory effect against the bacterial growth of *X. campestris* pv. *campestris*. The maximum inhibitory effect was shown by petals extract of *Tagetes erecta* (24.0 mm) and *Chrysanthemum coronarium* (23.50 mm). The test bacterium was less inhibited by petals extract of *Rosa damascena* (13.5 mm), *Acacia fernesiana* (11.5 mm), *Thevetia nerifolia* (11.5 mm), *Bombax malabaricum* (11.0 mm), *Anthocephalus cadamba* (10.5 mm) and *Lathyrus odoratus* (10.5 mm). The rest eleven plants samples did not show antibacterial effect against the test bacteria.

In general the Minimum Inhibitory Concentrations (MIC) of various plants extracts was observed 2.0%, while *Anthocephalus cadamba* and *Bombax malabaricum* showed 3.0% MIC. *Chrysanthemum coronarium* and *Tagetes erecta* showed 1.0% MIC for the test bacteria *X. campestris* pv. *campestris* (Tab. 2).

Table 1. Common names and families of plants used in experiment.

SN	Botanical name	Common name	Name of family	Distribution	Traditional uses of plants
1	*Acacia fernesiana* (L.)	Ghand Babul	*Mimosaceae*	Tropics	Flowers are a source of essential oil used in perfumery (Usher, 1971).
2	*Anthocephalus cadamba* (Mig.)	Kadam	*Rubiaceae*	Tropical Asia	The bark is used as a tonic and reduces fever (Usher, 1971).
3	*Bombax malabaricum* DC	Semul	*Bombacaceae*	Tropical Asia	The wood is a source of cellulose, resin; root and bark are used as an emetic. The gum is demulcent and used to treat diarrhea (Usher, 1971).
4	*Cassia fistula* (L.)	Amaltash	*Caesalpiniaceae*	Tropical Africa	The pulp of pods is used as a laxative (Usher, 1971).
5	*Cassia nodusa* (Ham.)	Gulabi Amaltash	*Caesalpiniaceae*	West Malaysia	The wood is used for posts and tool handles while roots are used as soap for washing clothes (Usher, 1971).
6	*Cassia siama* (Vahl.)	Siama	*Caesalpiniaceae*	India to Indonesia	The wood is used for heavy construction work, mine props and as a fuel (Usher, 1971).
7	*Callistemon lanceolatus*	Bottle Brush	*Myrtaceae*	Australlia, India	Leaves are a Tea substitute and have a delightfully refreshing flavour (Cribb, 1976), tan dye is obtained from the leaves (Grae, 1974).
8	*Cedrella toona* Roaxb.	Toon	*Meliaceae*	India to Australia	The flowers are the sources of red and yellow dyes and wood used for furniture, house building, tea chests, oil casks, and cigar boxes (Usher, 1971).
9	*Chrysanthemum coronarium* (L.)	Guldawadhi	*Compositae*	Asia, Africa, Mediterranean	The young seedlings are cooked as a vegetable in China and Japan (Usher, 1971).
10	*Delphinium ajacis* (L.)	Larkspur	*Ranunculaceae*	Europe, Mediterranean	A tincture of the dried ripe seeds is used

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Table 1-Contd....

					medicinally as a parasiticide (Usher, 1971).
11	*Jacranda mimosaefolia* (D.Don.)	Nili Gulmohar	*Bignoniaceae*	Tropical South America	The wood is used in general carpentry (Usher, 1971).
12	*Lagerstroemia flosreginae* (Retz.)	Jarul	*Lythraceae*	Malaysia	The wood is insect resistant and used for house building, flooring, bridges and railways sleepers (Usher, 1971).
13	*Lantana camera* (L.)	Ghaneri	*Verbenaceae*	Tropical America	A decoction of the leaves is used locally as a tonic and stimulant (Usher, 1971).
14	*Lathyrus odoratus* L.	Sweet Pea	*Leguminosae*	South Europe	An essential oil is extracted from flowers and used in perfumery (Usher, 1971).
15	*Nerium indicum* (Mill.)	Red Kaner	*Apocynaceae*	Tropical India	A poultice of root is used against ringworm; the flowers are used for perfume and produce a good honey (Usher, 1971).
16	*Nerium oleander* (L.)	White Kaner	*Apocynaceae*	Mediterranean	The roots are used in criminal poisoning and to exterminate rats (Usher, 1971).
17	*Nychtenthus arbortristis* (L.)	Har Sringar	*Verbenaceae*	India	The leaves yield a bright yellow dye (Usher, 1971).
18	*Rosa damascena* (Mill.)	Gulab	*Rosaceae*	Balkans and Asia Minor	The oil extracted from flowers is used in perfumery and for flavouring (Usher, 1971).
19	*Tagetes erecta* (L.)	Gendha	*Compositae*	Mexico, Old and New World, India	The flowers are used as source of yellow dye; decoction of flowers and leaves is used to treat intestinal worms, stomach upsets and to control menstruation (Usher, 1971).
20	*Thevetia nerifolia* (Juss.)	Pili Kaner	*Apocynaceae*	Tropical America, West Indies	The bark is used to reduce fevers (Usher, 1971).

Table 2. Anti-bacterial activity and minimum inhibitory concentrations of petal-extracts against *X. campestris* pv. *campestris*

SN	Plant species	Zone of inhibition (mm)*	Minimum inhibitory conc. (%)				
			0.25	0.5	1.0	2.0	3.0
1	*Acacia fernesiana* (L.)	11..50±1.24	+	+	+	-	-
2	*Anthocephalus cadamba* (Mig.)	10..50±2.15	+	+	+	+	-
3	*Bombax malabaricum* D.C.	11.00±1.78	+	+	+	+	-
4	*Cassia fistula* (L.)	-	NT	NT	NT	NT	NT
5	*Cassia nodusa* (Ham.)	-	NT	NT	NT	NT	NT
6	*Cassia siama* (Vahl.)	-	NT	NT	NT	NT	NT
7	*Callistemon lanceolatus*	-	NT	NT	NT	NT	NT
8	*Cedrella toona* Roxb.	-	NT	NT	NT	NT	NT
9	*Chrysanthemum coronarium* (L.)	23..50±0.35	+	+	-	-	-
10	*Delphinium ajacis*	-	NT	NT	NT	NT	NT
11	*Jacranda mimosaefolia* (D.Don.)	-	NT	NT	NT	NT	NT
12	*Lagerstroemia flos-reginae* (Retz.)	-	NT	NT	NT	NT	NT
13	*Lantana camera* (L.)	-	NT	NT	NT	NT	NT
14	*Lathyrus odoratus* L.	10..50±0.84	+	+	+	-	-
15	*Nerium indicum* (Mill.)	-	NT	NT	NT	NT	NT
16	*Nerium oleander* (L.)	-	NT	NT	NT	NT	NT
17	*Nychtenthus arbor-tristis* (L.)	-	NT	NT	NT	NT	NT
18	*Rosa damascena* (Mill.)	13..50±2.47	+	+	+	-	-
19	*Tagetes erecta* (L.)	24.0±0.26	+	+	-	-	-
20	*Thevetia nerifolia* (Juss.)	11.00±2.25	+	+	+	-	-

* Mean ±SD, NT= Not tested.

Discussion

Among the different plants screened, petal extracts of *Tagetes erecta* and *Chrysanthemum coronarium* showed maximum inhibitory activity against *X. campestris* pv. *campestris* (Tab. 2). Petal extract of *Tagetes erecta* was observed to show strong inhibitory effect against the bacterial growth of *X. campestris* (Tab. 2), which might be due to several antibacterial substances present in the plant sample, some phytochemicals. Various medicinal and antifungal properties of *Tagetes erecta* has been reported in literatures (Usher, 1971; Singh and Sharma, 1978; Baslas and Singh, 1981; Pandey, 1993), these correlate with our results. Petal extract of *Chrysanthemum coronarium* was observed to show inhibitory effect against the bacterial growth of *X. campestris*, which might be due various antimicrobial substances present in the plant sample, some phytochemicals such as coumarins, flavonoids and alcoholic soluble phenols. Petal extract of *Rosa damacena* was observed to show inhibitory effect against the test bacterial growth which might be due to various antimicrobial substances present in the plant sample, some phytochemicals like catechol, anthoxanthins, anthocynin, rutin, flavane and gallic acid, were found registered in literatures by several workers (Nicolls, 1970; Shekhawat and Prasad, 1971; Dixit *et al.*, 1975). The antimicrobial activities of plants studied have also been found registered in various literature i.e., *Acacia fernesiana* (Usher, 1971), *Anthocepholus cadamba* (Dilip and Bikash, 2004), *Thevetia* *nereifolia* (Usher, 1971; Pandey, 1993; Kurucheve *et al.,* 1997).

The test bacteria *X. campestris* pv. *campestris* was observed sensitive to a very low concentration (1.0%) of the aqueous extracts of *Chrysanthemum coronarium* and *Tagetes erecta*. The MIC was found slightly higher in case of petal extract of *Rosa damascena*, *Acacia fernesiana, Thevetia nerifolia* and *Lathyrus odoratus* against the test bacterium while *Anthocephalus cadamba* and *Bombax malabaricum* were observed to show inhibitory effect against the *X. campestris* pv. *campestris* at higher concentrations as compared to others tested plants samples (Tab. 2). The variations in the MIC might be due to differences in phytochemicals composition (Owuor *et al*., 1986; Toda *et al*., 1989).

Since the extracts of *Acacia fernesiana, Anthocephalus cadamba*, *Bombax malabaricum*, *Chrysanthemum coronarium* and *Lathyrus odoratus* used in this study have not been tested before as inhibitor of phytopathogenic bacteria, therefore, this may be a new report. The presence of various secondary metabolites such as alkaloids, quaternary alkaloids, coumarins, flavanoids, steroids/terpenoids, phenols etc., have been reported in the various plants extracts (Aswal *et al*., 1984; Abraham *et al*., 1986; Chopra *et al*., 1992) which may be responsible for the antibacterial properties of the plants studied.

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