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IN VITRO EFFICACY OF EIGHT PLANT EXTRACTS ON *Xanthomonas campestris* pv. *mangiferaeindicae* AFFECTING BACTERIAL BLACK SPOT OF MANGO.

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Abstract- This study was conducted to evaluate the *Xanthomonas campestris* pv. *mangiferaeindicae* (Xcm) which is causing bacterial black spot of mango, it is one of the most important bacterial disease and reduces mango crop yield in karnataka. The anti-Xcm activities of some plant crude extracts, by using solvents like petroleum ether, chloroform, methanol and water were tested for eight plants. The crude fruit extract of *Sapindus laurifolia* (15.0 mm) and leaf extracts of *Asclepias curassavica* (7.50 mm), *Helicteres isora* (7.0 mm), *Piper betel* (7.0mm), *Tamarindus indica* (5.0 mm), *Tridax procumbens* (5.0mm) and *Azadirachta indica* (7.0mm), exhibited greater antibacterial activities in Nutrient agar medium with MIC ranging from 3.0% to 12.0%. The test bacterium was less inhibited by leaf extract of *Coffee* leaf (4.0 mm), the observed synergy between plant extracts and plant pathogenic bacteria.
Key words- Mango, Plant extract, Solvents, *Xanthomonas campestris* pv. *mangiferaeindicae*

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Introduction

Bacterial black spot of mango caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (Xcm) is one of the most important bacterial disease causing mango crops in karnataka. Major strategies for disease management measures for mango fruit disease include extensive pre and post-harvest spraying with chemicals [6,11,21,24], and then adopted to reduce losses and prevent secondary infection in order to decrease epiphytic pathogen populations. In recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [20]. Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [10]. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails [19]. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides [25]. *In vitro* screening of plant extracts is an important first step in identifying plants with application potential in agriculture [22], The aim of this study was to screen and developing plant based formulations for plant disease management in vitro against Xcm.

Table 1- Used and properties of plants collected for anti-Xcm screening.

Botanical name	Family	Local name	Parts used
Helicteres isora	Sterculiaceae	Indian screw	Leaf
Piper betel	Piperaceae	Piper betel	Leaf
Asclepias curassavica	Asclepiadaceae	Milk weed	Leaf
Tamarindus indica	Fabaceae	Tamarind	Leaf
Tridax procumbens	Asteraceae	Tridax	Leaf
Azadirachta indica	Meliaceae	Neem	Leaf
Coffee sp.	Rubiaceae	Coffee	Leaf
Sapindus laurifolia	Sapindaceae	Soapnut tree	Fruit

Materials and Methods

Bacterial Cultures, Media and Growth Conditions

Four strains of Xcm were used: MYS-2, CTA- 6, CHK- 2 and KOL-4 (isolated and identified in the microbiology laboratory, Kuvempu university, Shankaraghatta) and strains were cultured in Yeast extract nutrient agar (YNA: yeast extract, 3 g; peptone, 5 g; sodium chloride, 5 g; agar, 20 g; in 1 l of distilled water) and incubated at 30^{0}C. Yeast extract nutrient broth (YNAB: yeast extract, 3 g; peptone, 5 g; sodium chloride, 5 g; in 1 l of distilled water) was used for liquid cultures.

Plant Materials and Preparation of Extracts

Fresh plant materials used in this study consisted of *Helicteres isora* (leaf), *Piper betel* (leaf), *Asclepias curassavica* (leaf), *Tamarindus indica* (leaf), *Tridax procumbens* (leaf), *Azadirachta indica* (leaf), *Coffee* leaf and fruit extract of *Sapindus laurifolia,* which were collected from Kuvempu university campus and Bhadra wildlife sanctuary, Western Ghats, Karnataka. The air-dried plant materials were grounded into fine powder and by using thimble of Soxhlet apparatus, crude was extracted with solvents like petroleum ether, chloroform, methanol and hot water. After filtration of total extracts, the extracts were evaporated to dry.

Anti-Xcm Activity Assay

Anti-Xcm activity of the different extracts was determined by cup diffusion method on nutrient agar medium (Anon, 1996). Wells are made in nutrient agar plate using cork borer (5 mm diameter) and inoculums containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension and 50 µl of the working solution of different plant extract, same volume of distilled water used for control and streptomycin was used as standard, then filled in the wells with the help of micropipette. Plates were incubated at 30°C for 24 h. After overnight incubation the plates were observed and measured for the zone of inhibition using scale and mean were recorded.

Determination of Minimum Bactericidal Concentration (MBC)

Freshly prepared nutrient broth was used as diluents. Crude extract was diluted by two fold serial dilution method. 50µl of the standard culture inoculums was added to each test tube except the negative control tube. All tubes were incubated at 37°C for 24 h. The tube content was subculture in fresh nutrient agar separately and MBC was determined as that showing no growth.

Results

The activity of the plant-extracts against the bacterial growth of *X. campestris* pv. *mangiferaeindicae* is presented in table 2. It was observed that out of 8 plants parts extracts tested, all plant extracts showed inhibitory effect against the bacterial growth of *Xcm* The maximum inhibitory effect was shown by fruit extract of *Sapindus laurifolia* (15.0 mm) and *Asclepias curassavica* (7.50 mm), *Helicteres isora* (7.0 mm), *Piper betel* (7.0mm), *Tamarindus indica* (5.0 mm), *Tridax procumbens* (5.0mm), *Azadirachta indica* (7.0mm), The test bacterium was less inhibited by leaf extract of *Coffee* leaf (4.0 mm), show antibacterial effect against the test bacteria. In general the Minimum Inhibitory Concentrations (MIC) of various plants extracts was observed 2.0% to 12%, showed for the test bacteria Xcm (Table. 3).

Table 2- Anti-Xcm activity of the crude plant extracts on mango bacterial black spot diseased strains.

Plant name	Zone of inhibition (mm)			
	Petroleum ether	Chloroform	Methanol	Water
Helicteres isora	5	-	7	NT
Piper betel	7	5	4	NT
Asclepias curassavica	-	7.5	5	NT
Tamarindus indica	-	-	-	5
Tridax procumbens	4	5	5	NT
Azadirachta indica	-	-	-	7
Coffee sp.	2	3	3	4
Sapindus laurifolia	15	9	13	NT
Control (Distilled water)	-	-	-	-
Streptomycin	20	20	20	20

Table 3- MIC and MBC of plant extracts on Xcm bacterial species (in µg/ml).

Plant name	Petroleum ether		Chloroform		Methanol		Water	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Helicteres isora	12	12	-	-	12	12	NT	NT
Piper betel	5	5	7	7	10	5	NT	NT
Asclepias curassavica	-	-	10	10	12	12	NT	NT
Tamarindus indica	NT	NT	NT	NT	NT	NT	3	3
Tridax procumbens	6	6	8	8	9	9	NT	NT
Azadirachta indica	NT	NT	NT	NT	NT	NT	2	2
Coffee sp.	12	12	9	9	8	4	4	4
Sapindus laurifolia	2	2	5	2	2	2	NT	NT

NT = Not Tested, - = Negative

Discussion

A coffee-leaf extract that has proved to be efficient in the control of several coffee and cotton diseases also exhibits potential for the control of bacterial spot in tomato. The disease severity was similar to the commercial product acibenzolar-S-methyl and the underlying molecular mechanisms involved in the disease control have been investigated in terms of the induction of plant genes [16].

The susceptibility to the plant extracts of *Clostridium* spp. Types A, B and F tested in this study did not significantly vary[7], the vegetative cells and spores of 62A and Okra isolates also had similar sensitivities to the plant extracts. It was also shown that some of the plant extracts had synergistic antibotulinal properties with $NaNO_2$. Such synergy was able to reduce the MIC of both plant extracts and $NaNO_2$. The combined antibotulinal efficacy of nutmeg, sage and clove extracts observed in the development of minimally processed meat products, particularly those with low levels of $NaNO_2$ (10 ppm) [7].

Management of plant diseases by application of plant products has previously been suggested [1, 2, 18], leaf extract of *D. metel* was found to be more effective in reducing the spread of sheath blight and bacterial blight diseases in rice [12]. Kumudini et al. [14] have reported that the leaf extract of D. metel exhibited 80% protection against the downy mildew pathogen, *Sclerospora graminicola*, and induced resistance in the highly susceptible HB3 cultivar of pearl millet. Various other plant species have been tested for the control of sheath and bacterial blight diseases. Ansari [3] suggested the use of *Trachispermum ammi* and *Ocimum* sp. to control sheath blight of rice without any harmful effects to the plant. Similarly, Gangopadhyay [8] reported the suppression of symptom development in turmeric (Curcuma longa) extract sprayed rice plants when inoculated with Xoo.

In the recent past, several plant species have been screened for antifungal activity and extracts/purified compounds from these plants were found to have a broad spectrum of antimicrobial activity and control pre-harvest [13,23,5] and post-harvest diseases of several plant species [17]. Datura metel completely inhibited in vitro conidial germination of *C. capsici* [9]. Kurucheve et al. [15] reported that extracts of *L. inermis* were inhibitory to *R. solani* and Bambawale et al. [4] reported that ethanol extracts of *L. inermis* were effective in control of the cotton pathogens *Alternaria macrospora, Myrothecium roridum* and *Xanthomonas compestris* in in vitro tests.

These reports drew our attention to develop plant-based products for control of bacterial black spot disease caused by Xcm in mango plants. In the present study, The activity of the plant-extracts against the bacterial growth of *X. campestris* pv. *mangiferaeindicae*. It was observed that out of 8 plants parts extracts tested, all plant extracts showed inhibitory effect against the bacterial growth of *Xcm* The maximum inhibitory effect was shown by fruit extract of *Sapindus laurifolia* and *Asclepias curassavica*, *Helicteres isora*, *Piper betel*, *Tamarindus indica*, *Tridax procumbens*, *Azadirachta indica,* The test bacterium was less inhibited by leaf extract of *Coffee* leaf, show antibacterial effect against the test bacteria.

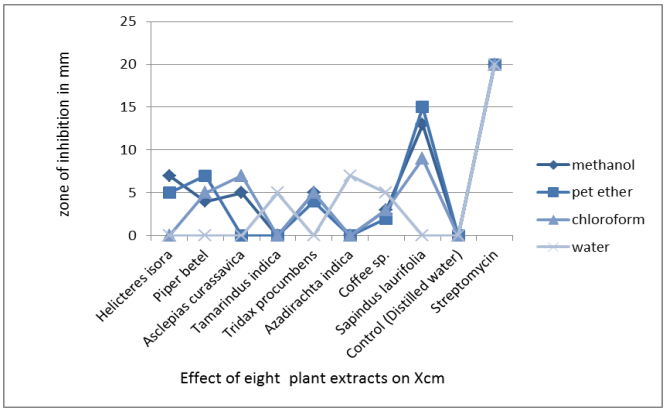

Fig. 1- Effect of eight plants extracts using different solvents against to Xcm.

Conclusions

Among the selected eight plant extracts, *Sapindus laurifolia* (15.0 mm) exhibited the highest bactericidal activity against Xcm. In addition, *Asclepias curassavica, Azadirachta indica* and *Helicteres isora* displayed strong bactericidal effect on Xcm under the same conditions. *Piper betel*, *Tamarindus indica*, *Tridax procumbens,* had a moderate and specific response in bacterial lethality, resulting in reductions in Xcm. These findings support the natural compounds may have a role as alternative bio controlling agents in plant disease management.

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