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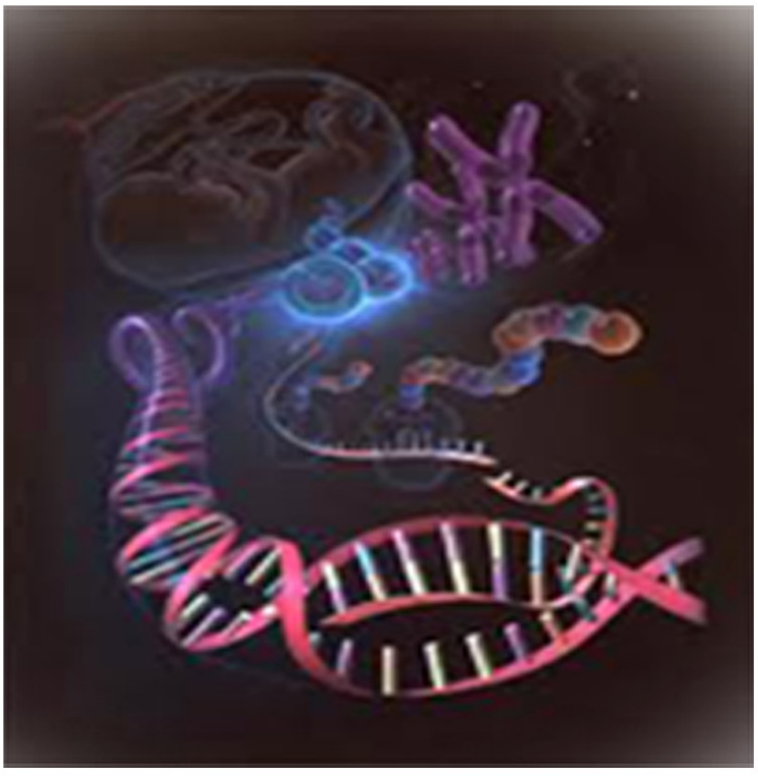

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Research Paper

ANTIBACTERIAL ACTIVITY OF *XYLARIA* SPECIES *IN VITRO* AGAINST *XANTHOMONAS CAMPESTRIS* PV. *MANGIFERAEINDICAE* ISOLATED FROM BACTERIAL BLACK SPOT OF MANGO FRUIT

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Macro fungi *Xylaria hypoxylon* and *Xylaria polymorpha* is common inhabitant of forest and woodland areas. 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. We aimed to determine the antibacterial potential of the petroleum ether, chloroform and ethanolicextracts obtained from *X. hypoxylon* and *X. polymorpha.* Extracts were tested for antibacterial activity against *Xanthomonas campestris* pv*. mangiferaeindicae* isolated from bacterial black spot of Mango fruit. The maximum inhibitory effect was shown by petroleum ether extract of *X. polymorpha.* The test bacterium was less inhibited by chloroform extract of *X. polymorpha*, shows antibacterial effect against the test bacteria.

Keywords: Bacterial black spot, Mango, *Xanthomonas campestris* pv*. mangiferaeindicae, Xylaria* spp.

INTRODUCTION

Macro fungi *Xylaria hypoxylon* (Figure 1) and *Xylaria polymorpha* (Figure 2) belongs to class Ascomycetes, commonly known as dead man's fingers. It is a common inhabitant of forest and woodland areas, usually growing from the bases of rotting or injured tree stumps and decaying wood (Hacioglu *et al*., 2011). 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. Mango is cultivated throughout the tropics as well as subtropical areas and is threatened by several destructive diseases (Pruvost *et al*., 2009). When the infection occurs after epiphytic spread of the pathogen, marginal necrosis of leaves and small black spots are common, and lesions, occasionally appear on fruits (Carlton *et al.*, 1998). When the disease symptoms increases lesions spread to stems, petioles and flowers, yield reductions can result from the reduced photosynthetic capacity of

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Figure 1: *Xylaria hypoxylon*

Figure 2: *Xylaria polymorpha*

infected foliage, leaf defoliation and flower abortion. Disease symptoms include necrosis of vegetative and flower buds and bud failure before bud break (Cozorla, 1998), then crop becomes infected with the bacteria.

Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action (Shihabudeen *et al.*, 2010). Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails (Satish *et al.*, 2007). 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 (Verma and Dubey, 1999). In this study, we aimed to determine the antibacterial potential of the petroleum ether, chloroform and ethanolic extracts obtained from *X. hypoxylon* and *X. polymorpha.* Extracts were tested for antibacterial activity against *Xanthomonas campestris* pv*. mangiferaeindicae* isolated from bacterial black spot of Mango fruit.

MATERIALS AND METHODS

Collection of Samples and Preparation of Extracts

The macro fungus *X. hypoxylon and X. polymorpha* were collected from Bhadra wildlife sanctuary in western ghats, Karnataka, which has a rich biodiversity hot spot. The collected *Xylaria* spp. samples were shade dried and crushed into fine powder by using electric blenders. The powders were stored in a clean and dry polythene bags. Then the 80 g powdered sample was extracted with 1 L solvents like petroleum ether, chloroform and methanol for 24 h with the help of soxhlet extraction equipment. Then the crude extract were dried and stored in an air tight container for further use (Hacioglu *et al*., 2011).

Xanthomonas campestris pv. *mangiferaeindicae* (*Xcm*) Culture, Media and Growth Conditions

The bacteria was isolated from bacterial black spot diseased fruit of mango in Karnataka and identified in the Department of Microbiology laboratory, Kuvempu university, Shankaraghatta and strains were cultured on 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°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.

Anti-*Xcm* Activity Assay

Anti-*Xcm* activity of the extracts of *X. hypoxylon* and *X. polymorpha* was determined by cup diffusion method on nutrient agar medium (Sathish *et al*., 1999). 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 *Xylaria* spp. 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.

RESULTS AND DISCUSSION

The activity of the *Xylaria* spp. extracts against the bacterial growth of *X. campestris* pv. *mangiferaeindicae* is presented in Table 1. It was observed that out of 6 crude extracts tested, all extracts showed inhibitory effect against the bacterial growth of *Xcm.* The maximum inhibitory effect was shown by petroleum ether extract of *X. polymorpha.* The test bacterium was less inhibited by chloroform extract of *X. polymorpha*, shows antibacterial effect against the test bacteria (Figure 3). In general the Minimum Inhibitory Concentrations (MIC) of various plants extracts was observed 2.0% to 12%, showed for the test bacteria *Xcm*.

In the current study, we evaluated the anti-*Xcm activity* of *X. hypoxylon* and *X. polymorpha* extracts, macro fungal species commonly available in Bhadra wildlife sanctuary in western ghats, Karnataka against *Xanthomonas campestris* pv*. mangiferae indicae* isolated from bacterial black spot of Mango fruit. Plants are the

Figure 3: Anti-*Xanthomonas campestris pv. mangiferaeindicae* Activity of *Xylaria* spp. Crude Extracts

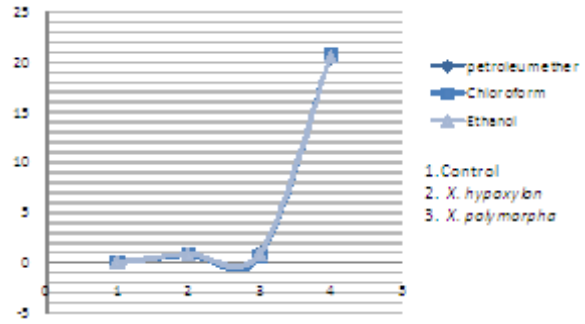

Table 1: Anti-*Xcm* Activity of *Xylaria* spp. Extract Measured in Zone of Inhibition (mm)

Species	Petroleum Ether	Chloroform	Ethanol
Control	0.0±0.0	0.0±0.0	0.0±0.0
X. *hypoxylon*	0.8±0.28	0.7±0.18	0.8±0.10
X. *polymorpha*	0.9±0.37	0.6±0.91	0.8±0.17
Streptomycin	20.50±0.12	20.71±0.68	20.58±0.46

Note: Values given are means of triplicates ± standard error.

richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trails. 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. Control of a number of plant diseases under commercial conditions has relied mainly on the application of a high number of fungicide sprays per season. Repeated application of some fungicides has caused residual toxicity, environmental pollution, phytotoxicity, an increase of resistant populations. For these reasons, the search for alternative control measures, such as biological control agents and plant extracts, has been challenging. Extracts which performed well in terms of inhibiting the growth of the plant pathogen in *in vitro* condition. Yenjit *et al.* (2010) findings are relevant for the potential enhancement of mango fruit quality by natural antifungal compounds from plants. Kagale *et al.* (2004) leaf extract of *D. metel* was found to be more effective in reducing the spread of sheath blight and bacterial blight diseases in rice. Ansari (1995) suggested the use of *Trachispermumammi* and *Ocimum* sp. to control sheath blight of rice without any harmful effects to the plant. Similarly, Gangopadhyay (1998) reported the suppression of symptom development in turmeric (*Curcuma longa*) extract sprayed rice plants when inoculated with *Xoo*. Bambawale *et al.* (1995) 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.

The ethanolic and water extracts of the mushrooms species especially *P. squarrosolus* inhibited the growth of majority of the isolates. Similar antimicrobial activities were reported (Lacobellies *et al*., 2005; Iwalokun *et al.*, 2007). This possibly indicated that the extracts possessed substances that can inhibit the growth of some microorganisms (Chika *et al.*, 2007) the observed inhibitory activities were more with the ethanolic extracts of the mushrooms species. Extracts of *P*. *squarrosolus* and *R*. *vesca* inhibited both Gram positive and negative bacteria as well as *C. albicans* suggesting broad–spectrum antimicrobial potentials. However, the inability of the extracts to inhibit the growth of *P. aeruginosa* could be that the organisms possess a mechanism for detoxifying the active components. Komemushi *et al.* (1996) observed inhibitory effect of *L. squarrosulus on* both gram-negative and gram positive bacteria.

The macrofungus differ significantly in their activity against tested microorganism (Yao and Moellering, 1995). Park, et al., (2005) reported that Liquid cultures of *Xylaria* sp. and griseofulvin exhibited potent broad-spectrum antifungal activity against rice blast, rice sheath blight, tomato graymold, wheat leaf rust, and barley powdery mildew. *Xylaria spp.* apparently has potential as a biological control agent for the control of various plant diseases. So our study reveals that the maximum inhibitory effect was shown by petroleum ether extract of *X. polymorpha.* The test bacterium was less inhibited by chloroform extract of *X. polymorpha*, shows antibacterial effect against the test bacteria.

CONCLUSION

Macro fungus and plants are the richest resource of drugs of traditional systems of medicine, these extracts exhibit antibacterial, antifungal and insecticidal properties under laboratory trails, and they have minimal environmental impact and danger to consumers in contrast to the synthetic pesticides. For these reasons, the search for alternative control measures, such as biological control agents and plant extracts, has been challenging. Extracts which performed well in terms of inhibiting the growth of the plant pathogen in *in vitro* condition. *Xylaria* spp. apparently has potential as a biological control agent for the control of various plant diseases and our study reveals that inhibitory effect was shown against the test bacteria.

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