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RELATIVE POTENTIAL OF DIFFERENT PLANT EXTRACTS AND ANTIBIOTICS AGAINST *XANTHOMONAS AXONOPODIS* PV*. MANGIFERAEINDICAE* CAUSING BACTERIAL LEAF SPOT OF MANGO IN LAB CONDITIONS

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A B S T R A C T

Bacterial leaf spot is a remerging disease in mango orchards of Pakistan and significantly lowers the yield every year. The present study was conducted to evaluate the potential of different plant extracts and antibiotics against *Xanthomonas axonopodis* pv*. mangiferaeindicae* in lab conditions. Aqueous extracts of five different plants i.e. Olive (*Olea europaea*), Neem (*Azadirachta indica*), Parthenium (*Parthenium hysterophorus*), Conocarpus (*Conocarpus erectus*) and Ginger (*Zingiber officinale*) were evaluated at 5, 10 and 15 % against the bacterial leaf spot of mango pathogen using disc sensitivity technique. Only Olive (*Olea europaea*) at 15 % concentration was found effective to inhibit bacterial growth. None of the other plant extract at any concentration was found effective. Three different antibiotics i.e. Gentamicin, Enrofloxacin and Validamycine were evaluated at 300, 600 and 1000 ppm against *Xanthomonas axonopodis* pv*. mangiferaeindicae*. Gentamicin at 1000 ppm was found most effective to inhibit bacterial growth. From the present study, it was found that Olive aqueous extract at 15 % and Gentamicin at 1000 ppm may be used to manage the disease after the field experimental trials.

Keywords: Mango (*Mangifera indica*). Antibiotics; Plant extracts. *Xanthomonas axonopodis* pv*. mangiferaeindicae*

INTRODUCTION

Mango (*Mangifera indica*) is grown in all parts of the world especially in tropical regions. Its fruit has a rich source of micronutrients, vitamins, carbohydrates, phenolic compounds, proteins, dietary fiber and fats (Tharanathan *et al*., 2006).

Bacterial leaf spot (BLS) is caused by *Xanthomonas axonopodis* pv*. mangiferaeindicae* (will be abbreviated as *Xam*) and has been reported in all those areas where mango is grown. It lowers 50% to 80% yield loss in different mango cultivars (Ploetz, 2003).

The pathogen enters the plant through natural opening and wounds and infects leaves, stem and fruit tissues. Circular raised water-soaked spots appear initially on the lower surface of the leave which turns brow and blackish with time. In favourable conditions, the lesions coalesce and form large necrotic patches. The bacteria may ooze from the infected tissues in humid conditions. (Gagnevin and Pruvost, 2001; Ploetz, 2003). In a severe case, the defoliation of leaves occurs. lenticels become weak with the fruit maturity and susceptibility to the disease increases significantly. On fruits, small irregular spots around the lenticels develop which turn black with time (Singh, 2018). The fruit drop is noticed in severe cases. longitudinally dark-colored cracks develop on the stem making it susceptible to wind breakage (Borkar and Yumlembam, 2016).

Chemicals are effective for disease management but have some serious issues like environmental toxicity and the development of resistance among the microbes (Anggriani *et al*., 2015). On the other hand, plant extracts are eco-friendly and have no residual effects. Plants have

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a rich different antimicrobial compound having significant efficacy against varieties of pathogens (Chethana *et al*., 2015).

The present study was conducted to evaluate the relative efficacy of different antibiotics and plant extracts against *Xam* in lab conditions.

MATERIAL AND METHOD

Collection of Diseased Samples: The disease samples of BLS of mango were collected from Ayub Agricultural Research Institute (AARI) Faisalabad and were wrapped in a paper envelope. Samples were placed in 4 ^{o}C in the lab for further studies.

Isolation of Pathogen: Leaves of BLS of mango were washed with tap water and then air-dried. Leaves were treated from 1% NaOCl for 1 minute to surface sterilize and then washed twice with autoclaved water and air-dried on filter paper. Disease samples were ground by pastel and mortar taking 1g leaves and 10 ml of autoclaved water. Serial dilution i.e.10^{-1} to 10^{-7} were made by adding the requisite amount of water.

For the pathogen isolation, 0.1 ml aliquot from each tube was taken with a sterilized pipette and poured in Petri dishes already containing nutrient glucose agar medium and was spread with the help of a sterilized spreader. Petri plates were wrapped and incubated for 24 hours at 25^{o}C for bacterial growth. After 24 hours, a single colony was picked by sterilized loop and streaked on NGA plates. The process was revised 2-3 times for purification and multiplied in liquid broth.

Evaluation plant extracts and antibiotics against BLS of mango: Fresh healthy leaves of Olive (*Olea europaea*), Neem (*Azadirachta indica*), Parthenium (*Parthenium hysterophorus*), Conocarpus (*Conocarpus erectus*) and Ginger (*Zingiber officinale*) were taken and thoroughly washed with running tap water and air-dried. 1g leaves were macerated by pastel and mortar in the presence of autoclaved water. The aliquot was filtered and was considered as a standard further dilution 5%, 10% and 15% were made by adding an adequate amount of water (Khan *et al*., 2000).

Four different commercially available clinical antibiotics i.e. Streptomycin sulfate, Oxytetracycline, Gentamicin and Validamycin were evaluated against the *Xam* at 300, 600 and 1000 ppm.

Lukewarm media was inoculated by pouring the nutrient broth having *Xam*. The media was poured into Petri dishes and allowed to solidify. Watman No. 1 filter papers were sterilized and discs of 6mm were made using a sterilized corn borer. The discs were dipped in test solutions (antibiotics and plant extracts) and were placed in the center of the petri dish already containing the bacterial culture. In control, the discs were dipped in sterilized water. The plates were wrapped and incubated at 25 ^{o}C. The data were recorded after 24, 48 and 72 hours by measuring the inhibition zones. The experiment was conducted in Completely Randomized Design (CRD) with three replications

STATISTICAL ANALYSIS

The data were statistically analyzed through Analysis of Variance (ANOVA) at 5% level of significance. The data were analyzed using "R" software. The treatment means were compared using Fisher's Least Significant Difference (LSD) test and represented by "Microsoft Office v. 2019" software.

RESULTS AND DISCUSSION

Efficacy of the plant extracts: Olive's extract at 15 % concentration was the only one that inhibited the bacterial growth of the *Xam* The extracts of Neem, Parthenium, Conocarpus and Ginger were found ineffective to inhibit bacterial growth at 5%, 10% and 15 % concentrations after 24, 48 and 72 hours of the application. After 24 hours, Olive's at 15 % concentration inhibited 1.72 Cm zone, however, a significant linear decrease in inhibition zone area was observed after 48 hours (1.4 Cm) and 72 hours (1.31 Cm) (Table 1).

The plant extract is a new hope to treat plant disease. Since the last two decades, many studies were conducted to explore the potential of different plant extracts against a variety of plant diseases (Alabouvette *et al*., 2006; Gurjar *et al*., 2012; Stangarlin *et al*., 2011). From the present study, it was revealed that among five plant extracts only the Olive at 15 % concentration was effective against the bacterial leaf spot pathogen. The answer of what is in the Olive which retarded the bacterial growth needs more study. Furthermore, in our study, we found that the inhibition zone area decreased with time. It suggests that Olive may have a bacteriostatic effect rather than the bactericidal. However, this area is open for future research. The other plant extracts in this study remained ineffective to inhibit bacterial growth, however, have been proved to be effective in other pathosystems (Hulloli *et al*., 1998; Meena and Gopalakrishnan, 2004; Shricharan and Sivabalan, 2020; Reddy *et al*., 2012; Kebede *et al*., 2013).

Efficacy of the antibiotics: After 24 hours at 300 ppm concentration, Gentamicin was most effective to inhibit bacterial growth as compared to Enrofloxacin and Validamycin. Validamycin was found least effective antibiotic to inhibit bacterial growth. Enrofloxacin was more effective as compared to Validamycin but was less to Gentamicin. At 600 ppm concentration, the efficacy of antibiotics increased as compared to the 300 ppm concentration to inhibit bacterial growth. At 1000 ppm, the effectiveness of the tested antibiotics to inhibit bacterial growth was more as compared to 300 and 600 ppm concentrations. Gentamicin was found most effective as compared to Validamycin and Enrofloxacin. Validamycin was the least effective to inhibit the growth of *Xanthomonas axonopodis* pv. *mangiferaeindicae.*

After 48 hours, the diameter of inhibition zones of all antibiotics increased as compared to 24 hours. At 300 ppm concentration, no difference between the efficacy of Gentamicin and Enrofloxacin was recorded. Validamycin was found least effective antibiotic to inhibit bacterial growth. At 600ppm, efficacy increased in all antibiotics. Gentamicin was found most effective as compared to Enrofloxacin and Validamycin. The efficacy of all antibiotics to inhibit bacterial growth were more at 1000 ppm as compared to 300 and 600 ppm concentration. Gentamicin was found most effective as compared to Enrofloxacin and Validamycin. Enrofloxacin was found effective than Validamycin but less than Gentamicin. In control treatment where only, sterilized water was applied no zone was recorded.

After 72 hours, the inhibition zones area significantly increased by all the antibiotics concerning 24 and 48 hours. At 300ppm, no difference in effectiveness was seen between the Gentamicin and Enrofloxacin. Validamycin was found least effective to inhibit bacterial growth. At 600ppm efficacy of all the antibiotics increased as compared to 300 ppm. Gentamicin was found most effective as compared to Enrofloxacin and Validamycin. Enrofloxacin was more effective than Validamycine. Validamycine was the least effective to inhibit bacterial growth. At 1000ppm, the effectiveness to inhibit the bacterial growth was more as compared to 300 ppm and 600 ppm. Gentamicin was found most effective as compared to Enrofloxacin and Validamycin. Enrofloxacin was also found effective than Validamycin but less than Gentamicin. In control treatment where only, sterilized water was applied no zone was recorded (Table 2).

The use of antibiotics on the plant to treat the diseases for more than 80 years results in the development of resistance in varieties of pathogens (Sundin and Wang, 2018). So, for successful bacterial disease management, new alternative bactericides must have to find. Recently many antibiotics like streptomycin and oxytetracycline have been efficiently used to treat many economical bacterial diseases of fruits, vegetables and field crops (Vidaver, 2002; McManus *et al*., 2002). From the present study, we found Gentamicin more effective antibiotics as compared to Enrofloxacin and Validamycin. Gentamicin is a broadspectrum antibiotic (Fitzgerald and Newquist, 2013) and is effective against both gram-negative and gram-positive bacterial. The antibiotic has bactericidal effective inhibits the protein synthesis by binding to 30 s ribosome (Hahn and Sarre, 1969). Indeed it is effective against *Xam* in controlled conditions, however, further study is needed to study its effectiveness in field conditions.

CONCLUSION

From the present study, it was found that extract from the Olive leaves at 15 % concentration and Gentamicin at 1000 ppm are effective to inhibit the *Xam* growth, so, these may be used to manage the bacterial leaf spot disease after the experimentation under the field conditions.

Table 1. Relative efficacy of plant extracts at different concentrations against colony growth of *Xanthomonas axonopodis* pv. *mangiferaeindicae*

	24 Hours			48 Hours			72 Hours		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
Olive	0.0 D	0.0 D	1.7 A	0.0 D	0.0 D	1.4 B	0.0 D	0.0 D	1.3 C
Neem	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D
Parthenium	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D
Conocarpus	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D
Ginger	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D
Control	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D	0.0 D
LSD	0.03								

Values having same alphabets are not significant to each others α = 0.05%

Table 2. Relative efficacy of bacteriocides at different concentrations against colony growth of *Xanthomonas axonopodis* pv. *mangiferaeindicae* in lab conditions

	24 Hours			48 Hours			72 Hours		
	300 ppm	600 ppm	1000 ppm	300 ppm	600 ppm	1000 ppm	300 ppm	600 ppm	1000 ppm
Gentamicin	1.5 H	2.0 EF	2.5 D	1.5 H	2.0 EF	2.6 C	1.5 H	2.1 E	3.0 A
Enrofloxacin	1.4 HI	1.9 F	2.4 D	1.5 H	1.9 F	2.4 D	1.5 H	2.0 EF	2.8 B
Validamycin	0.1 NO	0.8 L	1.2 JK	0.2 MN	0.9 L	1.3 IJ	0.3 M	1.1 K	1.7 G
Control	0.0 O	0.0 O	0.0 O	0.0 O	0.0 O	0.0 O	0.0 O	0.0 O	0.0 O
LSD	0.12								

Values having same alphabets are not significant to each othersα = 0.05%

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Contribution of Authors:

Muhammad E. U. Haq	:	Planned and supervised the study and wrote the "Results" section.
Muhammad U. Shahbaz	:	Recorded the experimental data.
Muhammad Kamran	:	Wrote the "Abstract" and "Material and Methods" Sections.
Muhammad J. Matloob	:	Wrote the "Introduction" section.
Wania Abrar	:	Performed the in vitro experiments.
Shaukat Ali	:	Wrote the "discussion" of the manuscript.
Abdul Rashid	:	Reviewed the manuscript technically and grammatically.
Muhammad A. Iqbal	:	Analyzed the experimental data.