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Original Research Article

EVALUATION OF ANTIBIOTICS TOWARDS THE MANAGEMENT OF BACTERIAL WILT OF ENSET (*Xanthomonas campestris* pv. *musacearum*)

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Abstract: Bacterial wilt is the major disease for enset and banana which causes huge yield loss. However, there are no recommended antibiotics for the disease. Hence, the objective of this study was to evaluate the efficacy of antibiotics against *Xanthomonas campestris pv. musacearum* (xcm) in vitro. Five antibiotics (namely, chloramphinicol (CAPH), streptomycin sulfate, tetracycline, amoxacillin and gentamycin) were tested in-vitro at three concentrations each (0.1, 0.5 and 1%). Sterilized distilled water was used as a control. Paper disk method was used for testing the sensitivity of the antibiotics. Their effectiveness were measured by the area of inhibition zone around the disk. Two pathogenic isolates (Gurage and Hagereselam isolates) were used. Completely randomized design (CRD) was implemented in factorial arrangement. All the in-vitro tested antibiotics had reduced the growth of bacterial culture significantly as compared to the control for both Gurage and Hagereselam isolates but some were more effective. Amoxacillin and tetracycline at 1 and 0.5% concentration are the most effective antibiotics in inhibiting xcm bacterium growth for Gurage isolate. Tetracycline and Chloramphinicol at 1 and 0.5% concentration are effective for Hagereselam isolates. This indicates that these antibiotics could be effective control agents for the disease. Therefore, they should be tested in-vivo.

Key Words: Antibiotics, Evaluation, inhibition zone, *Xanthomonas campestris* pv. *musacearum*

Introduction: In Ethiopia, *E. ventricosum* is arguably the most important crop contributing to food security and rural livelihoods for about 1/4 (20 million people) of the Country's population (Brandt *et al*., 1997). It is an endemic plant to Ethiopia. However, there are number of biotic and abiotic constraints that affect enset production in the country. The plant is drought resistant, high yielder and multipurpose crop. However, the farmers are majorly threatened by Enset bacterial wilt disease caused by *Xanthomonas campestris* pv. *musacearum* (xcm). The pathogen also attacks banana and wild enset (Tripathi *et al*., 2009). The disease is widely distributed and causes a serious disease

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loss on almost all enset growing regions (Weldemicheal *et al*., 2008).

The disease is ravaging enset plantations in the country. Cultural practices especially sanitation become an important management strategy. However, the disease spread in a short period by cultural practices and any contact to the diseased plant, owing to difficulty in eradicating the disease unless impressive management options are implemented. Once BWE occurs in a field, there is no remedy other than to cut down all infected plants, completely dig out the corm, and place the field under fallow or a prolonged crop rotation regime (Tripathi *et al*., 2009). Haile *et al*. (2014) also reported that the pathogen is variable in its pathogenicity.

So far, no antibiotics has been recommended against enset bacterial wilt. But, various *in-vitro* trials were conducted on antibiotics and plant extracts against other *X. campestris* pathovars that cause diseases in different crops. For example, streptomycin, oxytetracycline, chloroamphenicol and rifampicin were tested for the control of black rot of cauliflower caused by *X. campestris* pv. *campestris* and streptomycin was found to be the most effective antibiotics, giving 100% control followed by oxytetracycline (Lenka and Ram, 1997). Therefore, it is prudent to assess the potential of systemic antibiotics for the control of *X. campestris* pv. *musacearum*. Application of streptomycine sulphate and agrimycin-100 decrease the citrus canker disease caused by *X. campestris* pv. *citri* (Khan *et al*., 1992).

Knowledge of *in vitro* effect of antibiotics against the pathogen would provide the requisite information for further evaluation of their practical uses. The management of the disease basically depends on cultural practices like sanitation. The identification of effective antibiotics for the disease has been indispensable for effective management. Hence, this study was proposed to evaluate the efficacy of antibiotics against *X. campestris* pv. *musacearum in vitro*.

Materials and Methods

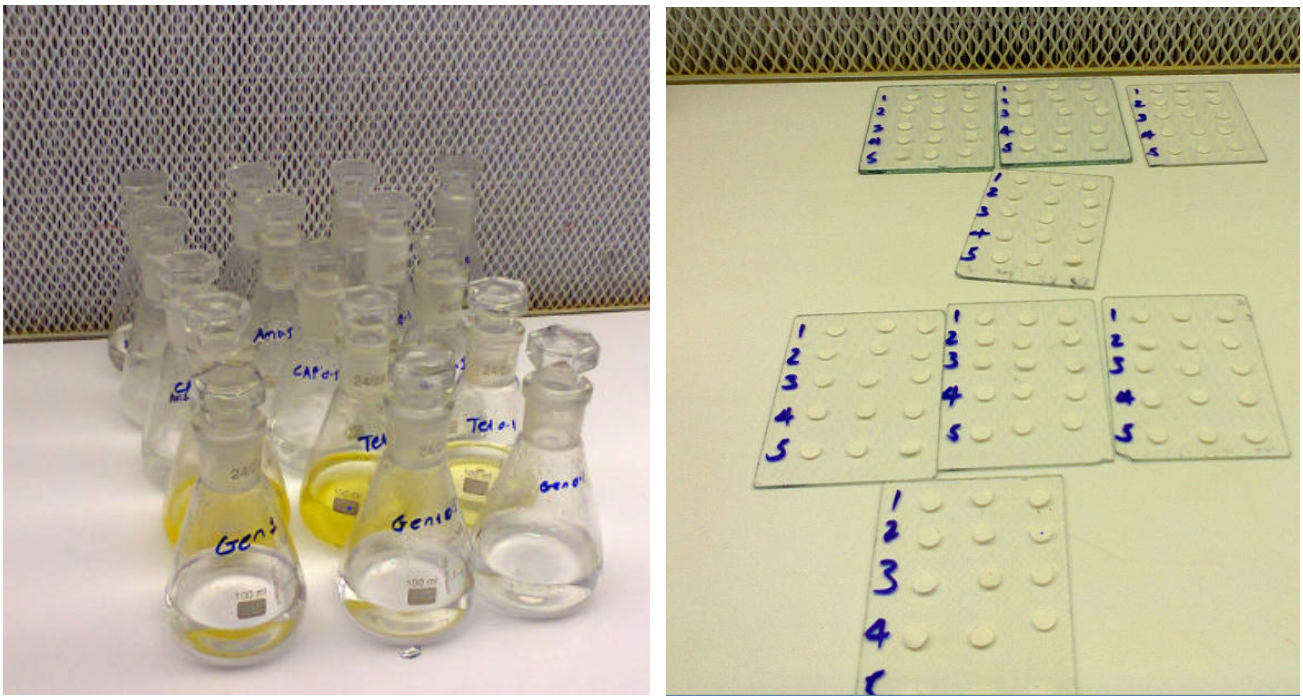
Bacterial Isolation: Fresh infected plants were collected from fields at Gurage and Hagereselam, then the bacterial ooze was collected and isolated by dilution plate technique. Hypersensitivity test was performed on tobacco plant while pathogenicity test was under taken by susceptible enset clone (Astara). The bacterium was purified by streaking method on Nutrient Glucose Agar (NGA) medium.

***In vitro* Evaluation of Antibiotics:** Sensitivity of *X. campestris* pv. *musacearum*, to five antibiotics were conducted by using paper disk diffusion assay techniques. Filter paper discs, 0.6 cm diameter, were cut with the help of cork borer and autoclaved at 180^{o}C for 1 hr. Solutions of five antibiotics namely chloramphinicol (CAPH), streptomycin sulfate, tetracycline, amoxacillin and gentamycin were prepared in three concentrations (0.1, 0.5 and 1%). Then 20 µl of each solution was dropped onto each disk and left for 10 minutes to evaporate. Only sterilized distilled water was used for the control disks. All the tested antibiotics were known to be effective against Gram-negative bacteria. The antibiotics were dissolved in sterile distilled water to achieve final concentrations of 0.1, 0.5 and 1%.

Bacterial suspension of *X. campestris* pv. *musacearum* (10^{8} cfu/ml by using spectrophotometer) was prepared as inoculum for this test. One milliliter of this suspension was poured into sterilized Petri dishes onto which about 20 ml of autoclaved YDC agar, cooled to about 50 ºC in a water bath, was poured. The Petri dishes were gently shaken to mix the bacterial cell suspension uniformly and allowed to solidify.

The paper discs were then placed onto the solidified nutrient agar in the petridishes containing the bacterium. The five antibiotics and the control were placed in each Petri dish. These Petri dishes were labeled and then incubated at 28 ^{o}C for 48 hours. This experiment was conducted for two isolates of Xcm, which were obtained from Gurage Zone

and Hagereselam (Sidama Zone). The experiment was laid out as Completely Randomized Design (CRD) in a factorial arrangement in the laboratory experiment. The experiment was conducted with three replications. The effects of different antibiotics and their concentrations were evaluated by measuring diameters of inhibition zones formed around the discs.



(A) (B)

Figure 1 Antibiotics dilution at different concentration (A) Antibiotics on paper disk (B)

Data Analysis: Collected data were subjected to the analysis of variance with SAS computer software version 9.2. Significant difference among treatment means was tested using LSD (Least Significant Difference) for evaluation of antibiotics at 1% level of significance.

Results and Discusion: The result from *in vitro* test revealed that all the five antibiotics (amoxacillin, tetracycline, chloramphinicol, streptomycin sulphate and gentamycin) tested were inhibited the growth of Xcm pathogen culture at all concentrations as compared to the control. However, the antibiotics showed variable reactions to the pathogen.

***In vitro* efficacy against antibiotics for Gurage isolate:** All the antibiotics at all concentrations were significantly ($p<0.001$) reduced the multiplication of *X. campestris* pv. *musacearum* for the Gurage isolate as compared to the control, but they varied greatly in their effects (Table 1). The diameter of inhibition zone ranged from 0.53 cm (for gentamycin at 0.1%) to 3.87 cm (for amoxacillin at 1%). The interaction effect between antibiotics and concentrations indicated that amoxacillin at a concentrations of 1%, tetracycline at 1%, amoxacillin at 0.5% and tetracycline at 0.5% concentrations were the most effective antibiotics in inhibiting the growth of the Gurage isolates of Xcm culture, in which the inhibition zones were 3.87, 3.20, 3.07 and 2.97 cm, respectively. Similarly, amoxacillin at 0.1%, CAPH at 1%, streptomycin sulphate at 1% concentrations were moderately effective and

had an inhibition zone of 2.7, 2.63 and 2.47cm, respectively. Thus, amoxacillin and tetracycline were found to be the most effective of all antibiotics at all concentrations against Xcm Gurage isolates.

On the other hand, gentamycin at 0.1%, gentamycin at 0.5%, CAPH at 0.1% and gentamycin at 1% were comparatively less effective in inhibiting the growth of the bacterial cultures, even if they were significantly inhibited the bacterial growth as compared to the control, with an inhibition zone of 0.53, 1.30, 1.43 and 1.47 cm, respectively. Gentamycin was found to be the least effective antibiotics in inhibiting the growth of Gurage isolates of Xcm with inhibition zone of 1.38 cm, followed by, streptomycin sulphate and CAPH which were comparatively moderately effective against Xcm with the diameter of inhibition zones of 2.52 and 2.58 cm, respectively.

Overall, the effect of all antibiotics in reducing bacterial growth significantly (<0.001) increased with increase in concentration. On contrary, there were bacterial growths around all control disks (no inhibition zones were observed) (Figure 2).

***In vitro* efficacy of antibiotics against Hagereselam isolates:** Analysis of variance (ANOVA) showed significant effects of antibiotics on the diameter of inhibition zone. Similar to the Gurage isolates, all the antibiotics at all concentrations significantly ($p<0.001$) reduced the multiplication of *X. campestris* pv. *musacearum* compared to the control against Hagereselam isolates, but they varied greatly with their efficacies. All the antibiotics showed lower to higher efficacies against Xcm. The maximum diameter (2.73 cm) of inhibition zone was observed due to tetracycline at a concentration of 1% and the minimum (0.40 cm) was for streptomycin sulphate at 0.1% concentrations. Tetracycline at rate 1%, CAPH at rate 0.5%, tetracycline at rate 0.5% and CAPH at rate 1% were the most effective antibiotics for inhibiting the growth of Xcm against Hagereselam isolates with inhibition zones of 2.73, 2.63, 2.47 and 2.47 cm, respectively.

Similarly, tetracycline and CAPH at a concentration of 0.1% and amoxacillin at 1% were moderately effective for the inhibition of the growth of these isolates and had inhibition zone diameters of 1.73, 1.73 and 1.63 cm, respectively. This result shows that tetracycline and chloramphinicol were found to be the most effective antibiotics for significantly inhibiting the growth of Xcm for Hagereselam isolate.

In contrast, streptomycin sulphate at 0.1% (0.40 cm), gentamycin at 0.1% (0.57 cm) and streptomycin sulphate at 0.5% (0.67 cm) were comparatively least effective for the inhibition of the growth of Xcm against Hagereselam isolates (Table 1). On the other hand, streptomycin sulphate was the least effective antibiotics for the inhibition of the growth of this pathogen at all concentrations with an average inhibition zone diameter of 0.61 cm, followed by gentamycin with 1.03 cm inhibition zone. This result revealed that the diameters of inhibition zones increased with the increase in concentrations of antibiotics. But the diameters of inhibition zones for all antibiotics at 0.1% concentrations were significantly ($p<0.001\%$) lower than 0.5 and 1% concentrations. No inhibition zone was recorded around the disks in all the control treatments (Figure 1).

Generally, these research results revealed that there was variation among the antibiotics in the inhibition of bacterial culture growths of Xcm for Gurage and Hagereselam isolates. All the antibiotics significantly reduced the multiplication of both Xcm isolates as compared to the control but they varied greatly in their effects. Similar findings were reported by Maher *et al*. (2005), against *Xanthomonas campestris* pathovars. Amoxacillin was found to be the most effective antibiotics in inhibiting the growth of Gurage isolate, but it was moderately effective against Hagereselam isolate. Tetracycline was effective against both isolates. In contrast, gentamycin and streptomycin sulphate were found to be the least effective

antibiotics against both isolates. For both isolates, as the concentration of antibiotics increased from 0.1 to 1%, the inhibition zones also increased. Maher *et al*. (2005) indicated that streptomycin sulphate at 0.1 and 1% concentrations was found to be the most effective against *X. campestris* pv. *citric* through in-vitro test. However, this research revealed that streptomycin sulphate was found to be least effective against Hagereselam isolate at all concentrations, while moderately effective at 1 and 0.5% concentrations and least effective at 0.1% concentration against Gurage isolate of Xcm pathogen.

Table 1. Inhibition zones due to antibiotics against the growth of Gurage and Hagereselam isolates of *X. campestris* pv. *musacearum*

Antibiotics	Inhibition Zones (cm)							
	Gurage Rate (%)				Hagereselam Rate (%)			
	1	0.5	0.1	Mean	1	0.5	0.1	Mean
Amoxacillin	3.87^{a}	3.07^{cd}	2.70^{cde}	3.21^{a}	1.63^{dc}	1.43^{e}	0.80^{g}	1.29^{b}
Tetracycline	3.20^{b}	2.97^{cbd}	2.23^{g}	2.83^{b}	2.73^{a}	2.47^{b}	1.73^{c}	2.31^{a}
Chloramphinicol	2.63^{fed}	2.30^{fg}	1.43^{ih}	2.12^{c}	2.47^{b}	2.63^{ba}	1.73^{c}	2.28^{a}
Strept. sulphate	2.47^{feg}	2.17^{g}	1.76^{g}	2.13^{c}	0.77^{g}	0.67^{hg}	0.40^{i}	0.61^{d}
Gentamycin	1.47^{ih}	1.30^{i}	0.53^{j}	1.10^{d}	1.47^{de}	1.00^{f}	0.57^{hi}	1.01^{c}
Control	0.00^{k}	0.00^{k}	0.00^{k}	0.00^{e}	0.00^{j}	0.00^{j}	0.00^{j}	0.00^{e}
Mean	2.27^{a}	1.97^{b}	1.44^{c}		1.51^{a}	1.37^{b}	0.87^{c}	
LSD (0.01%)	0.39	0.16	0.26		0.086	0.21	0.12	
CV (%)				9.28				7.51
SEM±				0.176				0.09

LSD, Least Significant Difference; CV, Coefficient of variation; SEM, Standard Error of Means; Means with different superscripts within the same column and class are statistically different at 1% level of significance.

Several experiments showed that *in vitro* and *in vivo* evaluation of antibiotics were effective for the control of different Xanthomonas species (Talwar *et al*., 1996). The current finding also revealed that *in vitro* evaluation of the five tested antibiotics showed significant inhibitory effect on both isolates of Xcm from Gurage and Hagereselam. A research by Singh *et al.* (2007) reported that streptomycin at 30 mg concentration was the most effective antibiotic, followed by chloramphenicol at 30 mg concentration, and gentamycin and tetracycline were intermediately effective against *Xanthomonas axonopodis* pv. *malvacearum*. Chloramphenicol was least effective for *in vitro* inhibition of *X. oryzae* pv. *oryzae* (Khan *et al.*, 2012). The current research also revealed that chloramphenicol was less effective against inhibition of Gurage isolates of Xcm but it was effective against Hagereselam isolate. Similarly, gentamycin was reported to be effective against some isolates of *X. maltophilia* (Khardori *et al.*, 1990). But in this research Gentamicin was less effective for both isolates of Xcm. Streptomycin and chloroamphenicol were also tested for the control of black rot of cauliflower caused by *Xanthomonas campestris* pv. *campestris* and they were effective in controlling the pathogen (Lenka and Ram, 1997). A report by Salid *et al*. (2013) indicated that in vitro evaluation of antibiotics was effective for bacterial blight of cotton. Tao *et al*. (2011) also reported that neomycin was effective for bacterial inhibition. Several researches also reported that botanicals

are effective for X. campestris pv. musacearum inhibition. For example Daniel and Getaneh (2015) reported that several botanical plant extracts are effective on in vitro inhibition of the pathogen.

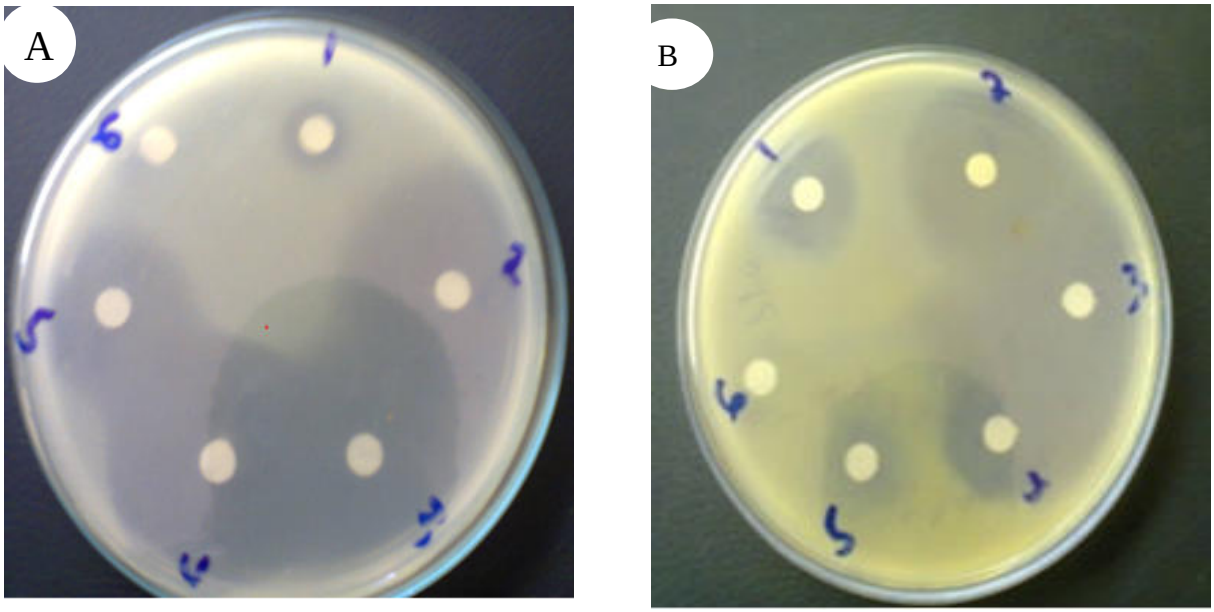


Figure 1. Inhibition zone of test antibiotics against *X. campestris* pv. *musacearum* isolates from Gurage (A) and Hagereselam (B) isolates

Conclusions and Recommendations: The data from *in vitro* evaluation of antibiotics against Xcm revealed the potentials of all the tested antibiotics in significantly inhibiting the growth of Xcm at all concentrations and regardless of sources of Xcm isolates. However, the efficacies varied significantly among antibiotics and the respective concentrations. For all antibiotics, the diameters of inhibition zones increased with the increase in concentration of the antibiotics.

Amoxacillin at all concentrations and tetracycline at 1 and 0.5% concentration were the most effective antibiotics aginst Gurage isolates of Xcm, while gentamycin was the least effective antibiotic at all concentrations. Chloramphinicol and tetracycline both at 1 and 0.5% were the most effective for inhibition of the bacterial culture against Hagereselam isolates, while streptomycin sulphate was the least effective antibiotic at all concentrations against the same isolates. Some of the *in vitro* tested antibiotics in the present study were effective against Xcm isolates. Therefore, their efficacies should be evaluated *in vivo* for the management of BWE under greenhouse and field conditions. Similarly, other additional systemic antibiotics should be evaluated *in vitro* and *in vivo* for their potentials in the integrated BWE management.

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