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Biocontrol of Phytopathogenic Bacteria Isolated from Drainage Water and Causing Bacterial Blight Disease

Safaa M. Ezzat

Central Laboratory for Environmental Quality Monitoring (CLEQM),
National Water Research Center (NWRC), Cairo, Egypt

Abstract: The present investigation was conducted to explore the incidence of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causing bacterial blight disease of rice crop in drainage water reused for irrigation and to evaluate the potentiality of using aqueous extracts from *Acacia nilotica* plant as natural bio-control for the pathogen and alternative to synthetic pesticides. Forty eight water samples were collected from El-Gharbia drain in two different seasons (summer and winter,2013) and assayed for traditional indicators of microbial water pollution (total and fecal coliforms). Results obtained and compared to WHO guidelines reported high incidence of fecal coliforms and categorized water reused from El-Gharbia drain as possibly suitable for irrigation of cereal, industrial and fodder crops under restrictions. Further bacteriological investigations revealed positive detection of *Xoo* in drainage water. Maximum peaks for the pathogen's occurrence were recorded in summer (71.40%) compared to winter season (28.60%) favored by the high temperature. The antagonistic effect (*in vitro*) of aqueous extracts (leaves and fruits) of *Acacia nilotica* plant was screened against *Xoo* isolates at different concentrations. The Pearson's correlation coefficient between the applied concentrations and inhibition zones showed significant correlation ($p<0.05$) for leaves extract and highly significant correlation ($p<0.01$) for fruits extract which was significantly comparable in its antagonistic effect to the synthetic bactericide "Streptocycline". The phytochemical analysis of the fruits extract revealed the presence of chemical compounds reported for their antimicrobial activity. The study concluded that removal of weeds from canals and ridges could effectively reduce the natural habitats for the pathogen and control its spreading through drainage and irrigation water. Aqueous extracts from *Acacia nilotica* were suggested as natural biocontrol for the pathogen either as protective agents for seeds and/or disinfectants in irrigation water. Future field studies are recommended for the development of these interesting extracts into an exploitable commercial product.

Key words: *Acacia nilotica* · Botanical extracts · Irrigation · Pesticides · Reused wastewater · *Xanthomonas oryzae* pv. *oryzae*.

INTRODUCTION

Microbial pollution of irrigation water has been recently recognized as a major threat not only for human health but also for crops yield and quality. Polluted water harbor a vast array of microbes that could infect susceptible plants causing diseases which result in losses up to 40% of global food production [1]. Nowadays, non-official reuse of drainage water in irrigation by individual farmers constitutes a real problem [2]. Plant nutrients and organic matter as well as drainage water availability are tempting factors for farmers to save their expenses [3]. In Egypt, there are two major crop seasons, namely, the winter season (November-May) and the summer season (May-October). The most important crops are wheat and berseem in winter, cotton, rice and maize in summer [4]. Rice (*Oryza sativa* L.) is an economically important cereal crop in Egypt. Rice production reached about 5.9 million ton in the year 2011-2012, with an increase of 4.2% than year 2010. Yield losses have been diminished through a series of preventive measures taken by the Egyptian authorities including, cultivation only once a year, planting resistant varieties, good fertilizers and pesticides management. Apart from these preventive

Corresponding Author: Safaa M. Ezzat, Central Laboratory for Environmental Quality Monitoring (CLEQM), National Water Research Center (NWRC), Cairo, Egypt.

measures, rice crop is still prone to a number of bacterial diseases, among which is bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae*.

The disease is devastating, found worldwide in temperate and tropical regions and can destroy up to 80% of the crop yield, particularly if infection takes place at early stage. The bacterium causing disease can be disseminated by irrigation water and can infect rice from seedling stage to mature plant. Infected plants produce fewer and lighter grains of poor quality. Seeds may be discolored and poorly filled with bacterial blight [5-7].

Modern agriculture farming has delivered over 500 compounds registered as synthetic pesticides to handle different plant diseases. Applications of such pesticides have been linked to a wide spectrum of human health hazards. The actual amount reaching the target organism is only about 0.1%, while the remaining bulk remains as residues in harvested crops and natural ecosystems [8]. Many pathogenic microorganisms have developed resistance against chemical pesticides. Traditional treatments such as application of copper compounds or antibiotics to control bacterial blight disease became ineffective due to extensive and prolonged use [9, 10]. These disadvantages are becoming fueling agents for searching new generation of pesticides which are more effective, eco-friendly, low-cost and easy to prepare [11].

Natural products derived from plants effectively meet these criteria. As a part of their defense system, many plants produce secondary metabolites which possess antimicrobial properties against pathogenic bacteria. These natural products are globally referred to as "Botanical pesticides" [12]. *Acacia nilotica* (L.) Delile is a locally widely distributed plant in Egypt. It grows on the banks of the canals crossing the delta and the Nile valley as well as Sinai, Red sea coastal region and in many deserts in Egypt [13]. It is commonly known as "Egyptian mimosa" or "Egyptian thorn" and belongs to family Leguminosae. In traditional medicine, it has been used to treat dysentery, diarrhea, hemorrhage, diabetes and has anti-inflammatory action [14, 15]. Its antibacterial activity against human pathogenic bacteria has been reported by Agunu *et al.* [16] and Dabur *et al.* [17], but reports concerning management of phytopathogenic bacteria are somehow limited and not studied so far. Inspired by pervious motivations, the present study was undertaken to explore the incidence of phytopathogenic bacteria (*Xanthomonas oryzae* pv. *oryzae*) causing bacterial blight disease of rice crop in drainage water reused for irrigation. An attempt has been made to evaluate the potentiality of using aqueous extracts from *Acacia nilotica* plant as natural bio-control for the pathogen and alternative to synthetic pesticides.

MATERIALS AND METHODS

Description of Study Area: For conducting this study, El-Gharbia drain was chosen as one of the most important and largest drainage systems located in the central part of the middle delta in Egypt (Fig.1). It originates in El- Gharbia Governorate north of Tanta city and extends through Kafr El-Sheik Governorate in north direction till the Mediterranean Sea at Balteem city. El-Gharbia drain was constructed at the period from 1930 to 1940. It has a total catchments area of about 700,000 faddans (one faddan=0.42ha) and length of about 71km. The catchments area is drained by pumping stations and discharges about 485.76 million m^3/ year to Lake Burullus and 842 million m^3/year to the Mediterranean Sea. Large areas are usually irrigated by the drainage water of that drain either officially or non officially. About 75% of the total drainage water of El-Gharbia drain comes from agricultural drainage, 4% from industrial drainage and 23% from domestic wastes[18,19 and 20]. This catchments area was selected based on the statistical background which ranked Kafer El-Sheik governorate as major rice cultivated area in Egypt. It accounts for about 40% of the total rice production capital in the country.

Water Samples: Sampling procedure was carried out in two different seasons (summer and winter 2013) according to Standard Methods for Examination of Water and Wastewater [21]. Eight major sites in El-Gharbia drainage system were selected as illustrated in Table 1. At least three independent samples were collected from each site in clean sterilized glass containers and stored in an iced cooler box and delivered immediately to the Central Laboratory for Environmental Quality Monitoring, National Water Research Center "CLEQM-NWRC" where it has been analyzed.

Bacteriological Analysis: Water samples were assayed for traditional indicators of microbial water pollution according to Standard Methods for Examination of Water and Wastewater [21]. For counting total coliforms (TC) and fecal coliforms (FC), the membrane filter technique was applied using a filtration system completed with stainless steel autoclavable manifold and oil-free "MILLIPORE" vacuum/pressure pump. Water samples were filtered through sterile, surface girded

Table 1: Sampling sites in El-Gharbia drainage system.

Location name	Description
Sagaaya	Sagaaya drain intake
Sematay	Sematay drain intake
P.S.3	Lifting drainage water to Gharbia main drain downstream Hamule P.S.
P.S.4	Lifting drainage water downstream P.S.3 & then to Gharbia main drain.
P.S.5	Lifting drainage water to Gharbia main drain downstream Sematay P.S.
P.S.6	Lifting drainage water to Gharbia main drain downstream P.S.5
Hamule P.S.	Mixing drainage water from Gharbia main drain downstream P.S. 6 to Tira canal
Tira canal	Upstream of reuse Hamule P.S.

P.S: Pump station

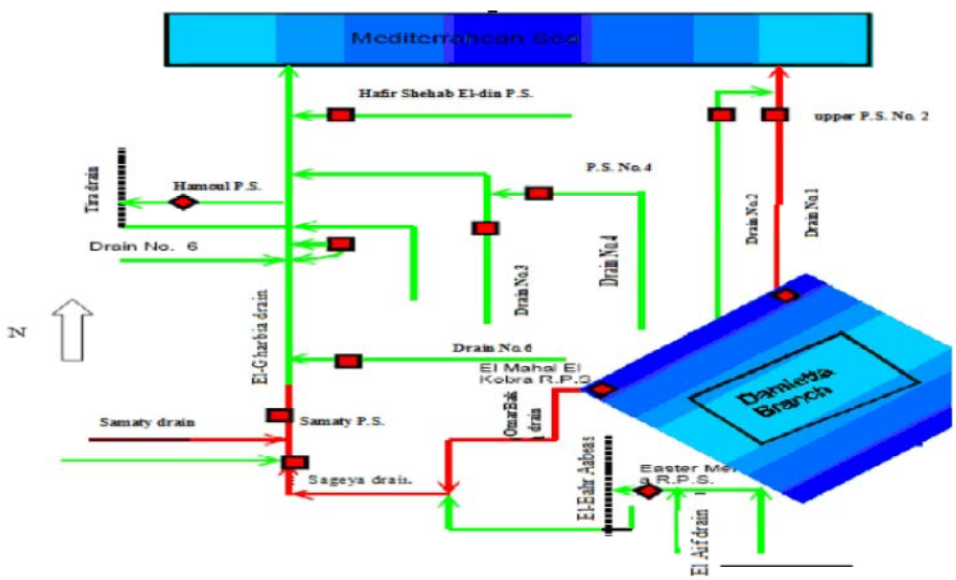

Fig. 1: Schematic diagram for El-Gharbia drainage system.

"SARTORIOUS" membrane of pore size 0.45 µm and diameter 47 mm, according to standard methods No. 9222B and 9222 D on M-Endo Agar LES and M-FC agar medium, respectively. All media used were obtained in a dehydrated form, Difco USA. Results were recorded as means of three independent samples and expressed in Colony Forming Unit (CFU/100 ml) using the following equation:

$$Colonies\ /100\ ml = \frac{counted\ \ colonies}{ml\ of\ sample\ \ filtered} \times 100$$

Isolation of Bacteria Causing Blight Disease: *Xanthomonas oryzae* pv. *oryzae* is a phytopathogenic bacteria causing blight disease in rice crop. Incidence of this plant pathogen was screened in water samples collected from El-Gharbia drain in two consecutive seasons (summer and winter) in the year 2013. The spread plate method was used for isolation in which, 0.1ml of undiluted and serially diluted water samples were spread plated onto Sucrose Peptone Agar (SPA) medium and the plates were incubated at 28°C for 24-48h. The rate of growth of xanthomonades on SPA is characteristic of the genus and the developed colonies are distinguishable by their typical yellow color and mucoid nature from other pathogens of related biochemical features. Suspected colonies developed from all tested water samples were randomly selected and re-streaked twice on Nutrient Agar plates to ensure purity. Pure isolates were maintained on Nutrient Agar slants at 4°C for further identification and confirmation tests [22, 23].

Microscopic Examination: To differentiate Gram positive and negative bacteria as well as shape and arrangement of cells, the bacterial cells were cultured overnight on Nutrient Agar plates. Each bacterial isolate was smeared with a drop of water on a cleaned, grease-free glass slide and air-dried. The prepared slides were Gram stained and examined under a light microscope with 100x oil immersion objectives and the picture was captured. The presence, shape and position of spores -if present- were examined microscopically by preparing smears of pure bacterial culture of 48h age stained by Malachite green for 5 minutes on water bath and safranin as a counter stain for 30 seconds. “Hiss staining technique” was used to detect the presence of capsules. The capsules -if present- is stained by pale blue color around bacterial cells stained by dark purple color. By applying “Hanging drop method”, motility of pure isolates were examined using a very small drop from a 24h liquid culture and examined microscopically using 40x objective [24, 25].

Biochemical and Physiological Tests: Basic microbiological techniques were employed to determine the ability of the bacterial isolates to utilize, hydrolyze and liquefy various chemical substances. In the present study, biochemical and physiological tests used to characterize *Xanthomonas oryzae* pv. *oryzae* included: KOH solubility, starch hydrolysis and gelatin liquefaction. Catalase, oxidase, urease and methyl red reactions as well as H_2S production. Acid from different carbohydrates *viz*: arabinose, sucrose, mannose, galactose, cellobiose, trehalose, rahmnose, mannitol, inulin, adonitol, inositol, salicin and sorbitol were also tested [24, 25].

Plant Samples: Samples of *Acacia nilotica* (leaves and fruits) proposed for screening the antibacterial activity of their extracts against *Xanthomonas oryzae* pv. *oryzae* were collected from specialized official botanic garden (Botanic Garden, Faculty of Pharmacy, Cairo University, Egypt).

Preparation of Plant Material: The selected parts (leaves and fruits) were washed thoroughly 2-3 times with running tap water and once with sterile distilled water and then subjected to oven drying at 40°C to constant moisture content as described by Al-Samarri *et al.* [11]. The dried samples were pulverized in an electric mixer and preserved away from moisture in closed labeled glass containers till investigation (Plate 1).

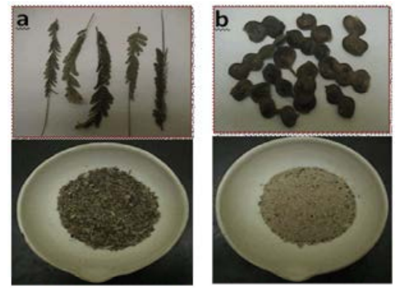

Plate 1: Dried and grinded (a) leaves and (b) fruits of *Acacia nilotica* plant.

Preparation of Plant Extracts: Aqueous extracts from *Acacia nilotica* (leaves and fruits) were prepared using water as a solvent. Water was chosen as an extraction medium because it is economically cost effective, easily available and eco-friendly technique. Up to ten grams of oven dried and power-pulverized plant materials were homogenized with 100 ml of sterile boiling distilled water. The flasks were vigorously shaken by vortex for 15 minutes and then allowed to stand at room temperature for 6 h. The obtained extracts were filtered through double layered muslin cloth at least twice and then through Whatman No. 1 filter papers and through 0.45 µm sterile membrane filters for extracts sterilization. The aqueous extracts were allowed to evaporate and the residues were dried to constant weight. The dried extracts were weighed and re-dissolved in appropriate volume of water to be used in the subsequent antibacterial activity assay [26, 27].

Antibacterial Activity Assay: The antibacterial activity of aqueous extracts from *Acacia nilotica* (leaves and fruits) was determined by agar well diffusion method [28]. Wells were made in nutrient agar plates containing 20 ml of agar media using sterile cork borer (7 mm). Inoculums containing 10^6 CFU ml^{-1} of bacteria (*Xanthomonas oryzae* pv. *oryzae*) were spread on the solid media with a sterile swab moistened with the bacterial suspension. The dried aqueous plant extracts were re-constituted in sterile distilled water to give different concentrations (100, 200, 300, 400 and 500 mg ml^{-1}) and 100 ul of each corresponding concentration were placed in the wells made in the inoculated plates. Also, 100 ul of sterile distilled water and antibiotic streptocycline (streptomycin sulphate and tetracycline) of 1mg ml^{-1} were placed separately in the wells to serve as negative and positive controls, respectively for comparative efficacy studies. The plates were incubated at 28°C for 24-48h and zones of inhibition -if any- around the wells were measured in millimeter (mm) and the minimum inhibitory concentration (MIC) was evaluated. Three replicates were maintained for each treatment.

Phytochemical Analysis: Preliminary Phytochemical analysis of plant extracts was conducted in the Central Laboratory of Desert Research Center following procedures of Harborne [29]. Screening of active secondary metabolites which could be responsible for antibacterial activity was recorded as presence or absence and included: Alkaloids, Flavonoids, Phenols, Steroids, Terpenoids, Saponins, Resins, Glycosides, Carbohydrates and Tannins.

Statistical Analysis: Results obtained were analyzed using SPSS software statistical program version 17. The correlation coefficient between different concentrations of plant extracts and corresponding mean inhibition zones were compared at significant levels 0.01 and 0.05 [30].

RESULTS AND DISCUSSION

Bacteriological Characteristics of Drainage Water Samples: Bacteriological evaluation of drainage water intended for reuse in irrigation is an important matter of concern regarding human health aspects as well as type, yield and quality of selected crop for cultivation. In the present study, traditional indicators of microbial water pollution (total and fecal coliforms) were used to assess the microbial quality and suitability of reused water from El Gharbia drain in irrigation purposes. Results presented in Table 2 declared obvious bacterial contamination in most of sampling locations. Total coliforms ranged between $65x10^2 – 29x10^5$ CFU/100 ml and $180x10^2 – 135x10^5$ CFU/100 ml, while fecal coliforms fluctuated between $23x10^2 – 6x10^5$ CFU/ 100 ml and $40x10^2 – 67x10^5$ CFU/ 100 ml in summer and winter seasons, respectively. The highest bacterial densities were always detected in winter season which could be attributed to accumulation of domestic wastes and agricultural runoff in drains during winter closure period that lowers water level leading to spontaneous increase in pollution load and decrease in dilution effect. Similar interpretation was also reported by Ezzat *et al.* [31] and El-Bahnasawy [32].

Irrigation of vegetables and fruits with raw wastewater can serve as a major pathway for bacteria, viruses and protozoa for both workers in agriculture and consumers of crops [33]. In this respect, the World Health Organization (WHO) recommended a maximum count of 1000 CFU/100 ml fecal coliforms as guideline for reused wastewater in irrigation (Table 3). Comparing our results given in table 2 to these guidelines, it is obviously clear that, water reused from El-Gharbia drain don't meet requirements for irrigating crops likely to be eaten uncooked. Meanwhile, irrigation of cereal, industrial and fodder crops is possibly suitable under restrictions. Our results are in harmony with those reported by El-Gammal *et al.* [34] who concluded that water in the concerned drain is highly contaminated by oxygen demanding substances and microbiological pollutants and that irrigation practices have high to moderate restrictions on reuse according to FAO and WHO guidelines.

Incidence of *Xanthomones oryzae* pv. *oryzae (Xoo)* in Drainage Water Samples: Bacterial blight is one of the most serious diseases that could Plough susceptible rice cultivars worldwide. In the present investigation, water samples collected from El-Gharbia drain were tested for the presence of the causative agent of the disease. Results demonstrated in Table 2 and illustrated by Fig. 2 revealed positive detection of *Xoo* isolates in drainage water. Maximum peaks were recorded in summer season (71.40 %) and ranged between 2-15 isolates. Meanwhile, in winter season detection was only limited to about 62% of the sampling locations and didn't exceed 9 isolates and represented 28.60% of the total isolates. The above results are supported by those of earlier investigators

Table 2: Bacteriological analysis of water samples collected from El-Gharbia drain in summer and winter seasons.

	Bacteriological Parameters					
	Tc[a]		Fc[b]		*Xoo* [c] No. of isolates	
	CFU/100 ml					
Sampling Locations	S[d]	W[e]	S	W	S	W
Segaaya	$29x10^5$	$135x10^5$	$6x10^5$	$67x10^5$	15	9
Sematay	$10x10^4$	$350x10^4$	$2x10^4$	$158x10^4$	12	5
P.S[f]. 3	$16x10^3$	$125x10^3$	$7x10^3$	$45x10^3$	3	ND[g]
P.S. 4	$140x10^3$	$380x10^3$	$80x10^3$	$210x10^3$	9	3
P.S. 5	$115x10^3$	$460x10^3$	$60x10^3$	$300x10^3$	7	3
P.S. 6	$72x10^3$	$138x10^3$	$13x10^3$	$47x10^3$	5	2
Hamule P.S.	$85x10^2$	$320x10^2$	$30x10^2$	$110x10^2$	2	ND
Tira canal	$65x10^2$	$180x10^2$	$23x10^2$	$40x10^2$	2	ND

Values are means of 3 independent samples (n=48)

[a]TC: total coliforms; [b]FC: fecal coliforms; [c]*Xoo*: *Xanthomonas oryzae* pv. *oryzae*; [d]S: summer; [e]W: winter; [f]P.S: pump station; [g]ND: not detected

Table 3: Recommended microbiological quality guidelines for wastewater reuse in agriculture [35].

Reuse conditions	Exposed group	Fecal coliforms (geometric mean no. per 100 ml)
Irrigation of crops likely to be eaten uncooked, sports fields, public parks	Workers, consumers, public	Less than or equal to1 000
Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees	Workers	No standard recommended

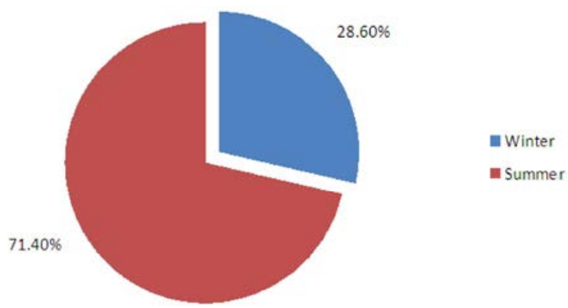

Fig. 2: Effect of seasonal variation on *Xanthomonas oryzae* pv. *oryzae* occurrence in drainage water.

Plate 2: (a) Streaked colonies of *Xoo* isolates on SPA plate. (b) Microscopic view of Gram stained *Xoo* cells (Magnification x 100).

who reported that the bacterium can be disseminated by irrigation and drainage water and that outbreaks of disease are more likely to occur during summer season (from June to September) favored by high temperatures [36-38].

Morphological and Biochemical Features of *Xoo* Isolates: out of forty eight processed water samples during two different seasons, 90 suspected bacterial isolates were

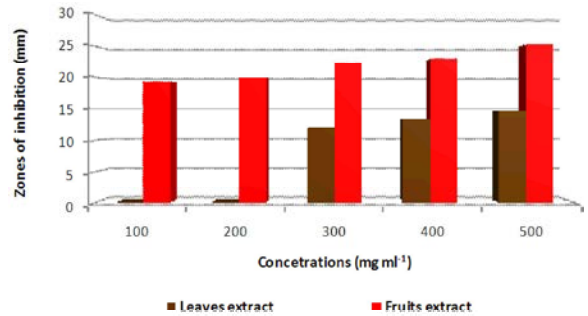

Fig. 3: Efficiency of aqueous extracts from *Acacia nilotica* at different concentrations.

recovered showing typical cultural characteristics of yellow, mucoid, round and entire colonies on SPA medium supplemented with 5% sucrose. About 77 (85.5%) of these isolates were microscopically and biochemically identified as *Xanthomonas oryzae* pv. *oryzae* as shown in plate 2 and given in Table 4. Strains described in the present study showed similarity with the literature description for *Xoo* given by Swings *et al.* [39]. The obtained results suggest that these stains appear to be phenotypically similar.

***In vitro* Inhibition Activity:** The antibacterial activity of natural aqueous extracts from *Acacia nilotica* (leaves and fruits) was evaluated -*in vitro*- against bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolated from reused drainage water. Results given in Table 5 and illustrated by Fig.3 demonstrate observable reduction in the pathogen's growth in terms of inhibition zones recorded. The aqueous fruits extract was more effective in inhibiting the growth of *Xoo* as compared to the leaves extract. Diameters of inhibition zones ranged between 11-15 mm for leaves extract and minimum inhibitory concentration (MIC) was 300mgml^{-1}. While for fruits extract, the zones ranged between a minimum of 19.0 mm and a maximum of 26.0 mm and the MIC was recorded at 100mgml^{-1}. Consistent observation in our study showed increase in inhibition activity with increasing concentrations. The same observation was also reported by Shehu *et al.* [40]. Plate 3 shows comparatively the efficiency of both extracts at different applied concentrations in relation to the negative control.

Table 4: Cultural, morphological and biochemical characteristics of isolated *Xanthomonas oryzae* pv. *oryzae*.

Characteristics	Result
Oxygen requirements	Strictly aerobic
Optimum growth temperature	25 - 30°C
Good growth on nutrient agar	+[a]
Mucoid growth on 5% sucrose	+
Shape of colony	Round, entire and mucoid
Pigmentation	Yellow endopigment
Gram reaction	G-ve
Cell morphology	Small rods
Cell arrangement	Single
Spore stain	-[b]
Capsule stain	-
Motility	+
KOH solubility, starch hydrolysis and gelatin liquefaction	+
Catalase reaction and H_2S production	+
Indole production and nitrate reduction	-
Oxidase, urease and methyl red reactions	-
Acid production from: arabinose, sucrose, mannose, galactose, cellobiose and trehalose	+
Acid production from: rahmnose, mannitol, inulin, adonitol, inositol, salicin and sorbitol	-

[a]+: Positive result; [b]-: Negative result

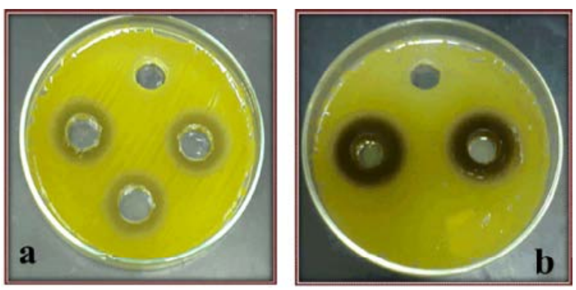

Plate 3: Antibacterial activity assay of *Acacia nilotica* (a) leaves extract (b) fruits extract.

As given in Table 6, the Pearson's correlation coefficient between the different concentrations of leaves extract and inhibition zones showed significant correlation ($p<0.05$) at 0.92. While for fruits extract, highly significant correlation ($p<0.01$) at 0.98 was observed. The inhibition zones developed due to the antibacterial activity of both extracts were also significantly correlated ($p<0.05$). The potential of antibacterial activity of aqueous fruits extract indicated that it is significantly comparable in its antagonistic effect to the synthetic standard bactericide "streptocycline". Although extracts from *Acacia nilotica* plant have been reported and recommended extensively as successful antimicrobial agents against human pathogens [16,17,41,42], yet its potential as botanical pesticide has not been exploited enough so far compared to other plants. In view of limited and available literature survey in this area, our results agree with those reported by Mahesh and Satish [43], Rajvaidhya *et al.* [44] and Fatima *et al.* [45]. These authors recorded significant antibacterial activity of *Acacia nilotica* extracts (leaf, bark and root) against several *Xanthomonas* pathovars associated with angular leaf spot of cotton, common blight of bean and bacterial spot of tomato. Further support to our data was elucidated through a recent study conducted by Kavitha and Satish [9], who proposed the use of leaf extracts from *Acacia nilotica* in management of bacterial blight disease of rice crop.

Preliminary Phytochemical Screening: Based on results obtained from antibacterial activity assay in this study, extracts (leaves and fruits) from *Acacia nilotica* were subjected for further chemical analysis to identify the possible effective compounds presumably responsible for such activity.

Results presented in Table 7 revealed the presence of nine broad chemical groups in the fruits extract. These groups included: Alkaloids, Flavonoids, Phenols, Steroids, Terpenoids, Saponins, Glycosides, Carbohydrates and Tannins. Meanwhile, only six groups were detected positive in the leaves extract and included: Alkaloids, Flavonoids, Phenols, Steroids, Terpenoids and Tannins. It seems obvious that the fruits extract is much rich in active compounds. This could interpret its higher antibacterial activity against the plant pathogen compared to that of leaves and supports its highly significant effect concluded from statistical analysis. Parallel to the above mentioned results, several studies reported the antimicrobial effect of these natural compounds particularly, flavonoids, phenols, tannins and steroids [9, 10, 44]. These compounds are often synthesized by

Table 5: Antibacterial activity assay of aqueous extracts from *Acacia nilotica* plant against *Xanthomonas oryzae* pv. *oryzae.*

	Zone of inhibition (mm)					
	Leaves extract			Fruits extract		
Concentration (mg ml^{-1})	Min.	Max.	Mean ± SE	Min.	Max.	Mean ± SE
100	0.0	0.0	0.0 ± 0.0	19.0	20.0	19.33 ± 0.33
200	0.0	0.0	0.0 ± 0.0	19.0	21.0	20.0 ± 0.57
300	11.0	13.0	12.0 ± 0.57	22.0	23.0	22.33 ± 0.33
400	13.0	14.0	13.33 ± 0.33	22.0	24.0	23.0 ± 0.57
500	14.0	15.0	14.66 ± 0.33	24.0	26.0	25.33 ± 0.66
Control [a]	0.0	0.0	0.0 ± 0.0	0.0	0.0	0.0 ± 0.0
Streptocycline [b]	25.0	27.0	26.0 ± 0.50	25.0	27.0	26.0 ± 0.50

Values are mean inhibition zones (mm) ± standard error of 3 independent replicates

[a] negative control (sterilized distilled water); [b] positive control (standard antibiotic 1mg ml^{-1})

Table 6: Correlation coefficient between different concentrations of leaves and fruits extracts from *Acacia nilotica* and corresponding zones of inhibition.

		Zones of inhibition	
Variable	Concentrations	Leaves	Fruits
Concentrations	1.00		
Zones of inhibition (Leaves)	0.916*	1.00	
Zones of inhibition (Fruits)	0.982**	0.931*	1.00

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 7: Preliminary phytochemical analysis of aqueous extracts from *Acacia nilotica* plant.

Phytochemical compound	Leaves extract	Fruits extract
Alkaloids	+[a]	+
Flavonoids	+	+
Phenols	+	+
Steroids	+	+
Terpenoids	+	+
Saponins	-[b]	+
Resins	-	-
Glycosides	-	+
Carbohydrates	-	+
Tannins	+	+

[a]+: Present; [b]-: Absent

plants in response to microbial infections and can successfully serve in plant defense mechanism against pathogenic microorganisms [12]. In general, it can't be excluded that in addition to the main compounds; other minor components might be present and have a significant contribution to the extracts activity. Thus, it is more advantageous to examine the activity of aqueous total extract rather than each compound individually. This will expectedly show higher activity due to synergistic action [46, 47]. Results obtained throughout the present investigation sums up the importance of reused waste water quality studies and present natural botanical pesticides as a more realistic, cost effective and ecologically sound method in plant disease management strategies.

CONCLUSIONS AND RECOMMENDATIONS

The present study is expected to provide first hand information on the occurrence of bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* in drainage water reused for irrigation of rice crop. The developed data could be a starting guide for decision makers to control spreading of the disease. Removal of weeds from canals and ridges would effectively reduce the natural habitats for the pathogen dispersal through drainage and irrigation water. We also demonstrate the antibacterial activity of *Acacia nilotica* plant extracts (leaves and fruits) as natural bio-control and highlight their strong *in vitro* inhibition activity against the pathogen. As the bacterium is known to be transmitted through seeds, the study suggests the application of plant extracts as seed protecting agents and/or as disinfectants in irrigation water before and after transplanting the seedlings. Future field studies are recommended for the development of these interesting extracts into an exploitable commercial product.

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