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Eichhornia crassipes from wastes to valuable products in water purification and influence on growth and impregnability in overwhelmed broiler chickens

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Abstract

Background: The implementation of green technologies is continually gaining attention worldwide and was considered for removing water pollutants and treating municipal water before its disposal.

Aim: Evaluation of the laboratory antimicrobial actions and chelating activities, and the field influence of *Eichhornia crassipes* on performance, biochemical and immunoglobulin concentrations, and intestinal microbiota in overwhelmed broiler chickens.

Methods: We assessed the laboratory antimicrobial actions of *E. crassipes* 1% suspension against bacterial (*Escherichia coli* O157: H7 and *Salmonella* Typhimurium) and fungal (*Aspergillus niger* and *Candida albicans*) microorganisms using a 96-well minimal inhibitory concentration, and the chelating activities of *E. crassipes* against calcium sulfate and copper sulfate. Also, we designed randomly four equal groups out of 200 1–day-old Ross® 308 chicks on a deep litter system. Three groups (G1, G2, and G3) were supplied daily with *E. crassipes* suspension of 1% from the third day until the end of the experiment, while the fourth group (G4) received non-treated tap water. Broilers of G1–3 were challenged with calcium sulfate (75 $mg.l^{-1}$), copper sulfate (200 $mg.l^{-1}$), and *S.* Typhimurium (1.5×10^6 $CFU.ml^{-1}$) polluted water respectively on the 7th, 14th, 21st, 28th, 35th days of age. We collected 1,914 samples by the end of the study, these samples included 90 *E. crassipes* pollutants and 480 *E. crassipes* microbial mixes, 192 sera, 192 intestinal swabs, and 960 tissues.

Results: *Eichhornia crassipes* treated water reveals highly significant ($p < 0.01$) improvements in water quality assessments and a highly significant ($p < 0.01$) increase in dissolved oxygen levels compared to tap water. *Eichhornia crassipes* 1% achieved a 100% adsorption capability for calcium and copper sulfate after 1-hour and 100% bactericidal (*E. coli* O157: H7 and *S.* Typhimurium) and fungicidal (*A. niger* and *C. albicans*) actions after 1, 2, 2, and 2-hours, respectively. Broilers treated with 1% *E. crassipes* revealed highly significant ($p < 0.01$) improvements in performance indices, carcasses characteristics, biochemical and immunological parameters, and highly significant ($p < 0.01$) decreases of cortisol hormone and bacteriological parameters in all treated broiler groups compared to the control.

Conclusion: *Eichhornia crassipes* 1% reveals a significant improvement in drinking water quality, as well as produces high adsorptive and antimicrobial actions. *Eichhornia crassipes* 1% improved performance traits, carcass quality, and intestinal microbiota in overwhelmed broilers.

Keywords: Antimicrobial, Broiler chickens, *Eichhornia crassipes*, Immunity, Performance.

Introduction

Water pollution is a major worldwide reproducible problem with urbanization and industrial growth. Water quality has been used to point out the physicochemical characteristics to meet suitability for various uses (Li and Qian, 2018a). Water quality is influenced by numerous factors including source geology, meteorological conditions, topography, seasonal variations, and biological considerations (Li *et al*., 2017a). Clean and palatable water with freedom from disease-producing agents has been the main demand for livestock production (Tian and Wu, 2019). Water is negatively affected by populations' activities and becomes polluted with chemicals and infectious/zoonotic pathogens including bacteria (Soliman *et al*., 2009a) such as *Escherichia coli*, *Salmonella*, *Leptospira*, and *Listeria*, protozoa (Soliman *et al.*, 2009b) such as *Giardia* and *Eimeria*, and parasitic agents such as *Echinococcus*, *Schistosoma*, and *Faschiola*. Biosecurity pillars have

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included water quality to provide birds with excellent/satisfactory water (Su *et al*., 2019).

Water purification has been performed using various methods such as photocatalysis (Raval *et al*., 2016), biochar (Palansooriya *et al*., 2020), sludge materials (Anastopoulos *et al*., 2017), ion exchange (Szczepanik, 2017), and eco-friendly byproducts as clay (Soliman *et al*., 2021d) and *Oreochromis niloticus* bone (Soliman *et al*., 2022). Developing eco-friendly, economical, and less destructive alternatives (Phytoremediation) for the handling of water pollutants is a worldwide interest (Shah, 2010). These eco-friendly means must possess higher absorption capabilities in short contact time and accumulation actions for longer periods (Mudgal et al., 2010). Aquatic plants such as *Eichhornia crassipes* are characterized by high invasiveness, wide spreading (tropical and sub-tropical countries), floating freely, and can grow up to 1 m above the water's surface (Pellegrini *et al.*, 2018). It is known as a fast-growing runner plant and its seeds can remain viable for 28 years (*Sarika et al., 2014)*.

Eichhornia crassipes usually survive in an ambient temperature of 25°C–30°C (12°C: 35°C), pH of 5.0: 7.5, and salinity of low than 15%, which above contributes to chlorosis and death (Kong *et al*., 2014). It has been recognized as an ecological plague in Egypt from 1879 to 1892 and contributed to biomass suffocation, diminishing fish reservoirs, and negatively influencing the economy (Twongo, 2019). *Eichhornia crassipes* has high absorbing power and thus has been used in wastewater purification. *Eichhornia crassipes* have been used in numerous fields such as braiding material and fiber source in India, Thailand, and Vietnam, biogas production, sludge field, adsorbent (Abdel Shafy *et al*., 2016), bio-cleaning (Ansari *et al*., 2014*)*, food source, small scale paper production, carotene-based table vegetable, dermatological medicinal applications in horses, sustainable source of oxygen, and bio-herbicidal agent through the production of hydrogen peroxide and inhibition of the soluble peroxidase activities (Chai *et al.,* 2013*)*.

Here, in this study, we evaluated the laboratory antimicrobial actions of the *E. crassipes* 1% suspension against bacterial (*E. coli* O1527: H7 1.7×10^{11} CFU. ml^{-1} and *Salmonella* Typhimurium 1.5×10^{6} CFU.ml^{-1}) and mycotic (*Aspergillus niger* 2.5×10^{8} CFU.ml^{-1} and *Candida albicans* 2.5×10^{8} CFU. ml^{-1}) microorganisms, and the chelating activity against inorganic (calcium sulfate, 75 $mg.l^{-1}$) and heavy metals (Copper sulfate, 200 $mg.l^{-1}$) water pollutants. We also evaluated the field influence of the *E. crassipes* 1% suspension on growth traits, some biochemical parameters, immunoglobulin concentrations, some antioxidant enzymatic activity, cortisol hormone levels, and intestinal microbiota in broiler chickens challenged once weekly with polluted/contaminated (calcium sulfate; 75 $mg.l^{-1}$, copper sulfate; 200 $mg.l^{-1}$, and *S.* Typhimurium; 1.5×10^{6} CFU.ml^{-1}) water.

Materials and Methods

Study location and duration

The laboratory (antimicrobial and chelating activities) trials were run in the Veterinary Public Health Department Laboratory from June to July 2022 and the field experiment was run in the broilers' units at Suez Canal University from August to September 2022. Water quality, production traits, carcass quality, and biochemical and bacteriological examinations were run in the Veterinary Public Health Department Laboratory. Water electrolytes, antioxidant enzymes, and cortisol and immunoglobulin concentrations were run in the University Hospital Laboratories. Heavy metal analysis was run in the Toxicology Laboratory at Suez Canal University.

Eichhornia crassipes preparation

The procedures were run after Sarkar *et al*. (2017) with some modifications. *Eichhornia crassipes* leaves were obtained from the Ismailia canal in polyethylene bags. The leaves were washed with de-ionized water four to five times, cut into pieces, sun-dried for 24–72 hours, and minced in a mortar (Granite-Rock). The minced powder was extra-dried in a hot air oven (Daihan®, Indonesia) at 80°C/24–48 hours. The powder was stored in a dark bottle.

Pollutant suspensions preparation

Inorganic calcium sulfate dihydrate (Spectrum®, United States) and organic copper sulfate (HiMedia®, India) powders were purchased. Calcium sulfate was dissolved at a rate of 75 $mg.l^{-1}$ in distilled water (Total hardness = 2,100 $mg.l^{-1}$ $CaCO_3$; MPL < 75 $mg.l^{-1}$ and calcium hardness = 1,600 $mg.l^{-1}$ $CaCO_3$; MPL < 60 $mg.l^{-1}$). Copper sulfate was dissolved at a rate of 200 $mg.l^{-1}$ in distilled water (Copper = 5 $mg.l^{-1}$; MPL = 1.3 $mg.l^{-1}$).

Propagation of bacterial and fungal isolates

The bacterial isolates were propagated after Soliman *et al*. (2021b, 2021c). *Escherichia coli* O157: H7 suspension (2.0×10^{4} CFU.ml^{-1}) was propagated into Mac-Conkey broth (Oxoid™) at 44°C/24 hours, eosin methylene blue agar (Oxoid™) at 37°C/24 hours, and tryptone soya broth (Oxoid™) providing 1.7×10^{11} CFU. ml^{-1} suspension. Lyophilized *S.* Typhimurium (3.4×10^{2} CFU) was propagated into Rappaport-Vassiliadis broth (Oxoid™) at 37°C/24 hours, xylose lysine deoxycholate agar (Oxoid™) at 37°C/24 hours, and tryptone soya broth providing 1.5×10^{6} CFU.ml^{-1} suspension.

The fungal isolates were propagated following Soliman *et al*. (2021b). *Aspergillus niger* and *C. albicans* clinical isolates were propagated into sabouraud dextrose broth (SDB; HIMEDIA®) at 37°C/24–72 hours, sabouraud dextrose agar (SDA; Oxoid™) at 37°C/24 hours, identified with lactophenol cotton blue stain (Hardy Diagnostics®), and resuspended in SDB broth, providing 2.5×10^{8} CFU.ml^{-1} suspensions.

In-vitro antimicrobial activities of E. crassipes

A 96-well minimal inhibitory concentration technique was run to evaluate the antimicrobial efficacy of

E. crassipes 1% on *E. coli* O157: H7 (1.7×10^{11} CFU. ml^{-1}), *S.* Typhimurium (1.5×10^{6} CFU.ml^{-1}), *A. niger* (2.5×10^{8} CFU. ml^{-1}), and *C. albicans* (2.5×10^{8} CFU. ml^{-1}) after Elshikh *et al*. (2016) with a few modifications. The 96 wells were inoculated with 100 µl of *E. crassipes* 1% suspension. Wells were inoculated with 10 µl from microbial suspensions as follows: *E. coli* O157: H7 into C1–3 (*n* = 24 wells), *S.* Typhimurium into C4–6 (*n* = 24 wells), *A. niger* into C7–9 (*n* = 24 wells), and *C. albicans* into C10–12 (*n* = 24 wells).

After contact times (0.25, 0.5, 1.0, 2.0, and 4.0 hours), 10 µl of the mixes were added into 10 ml of buffered peptone water (Oxoid™). The tubes were mixed at 37°C/2 hours (Elshikh *et al*., 2016). 10 µl were dropped onto eosin methylene blue, XLD, and SDA agar at 37°C/24 hours. The specific colonies were counted (Quebec Darkfield Colony Counter). The antimicrobial efficacies (%) of *E. crassipes* 1% were calculated by proportionating the surviving colonies to the initial counts as follows:

$$\frac{\text{The initial microbial count–Number of Surviving organisms}}{\text{The initial microbial count}} \times 100$$

In-vitro chelating activities of E. crassipes

1 ml of *E. crassipes* 1% suspension (1 $g.l^{-1}$) was dispensed into each of three triplets of conical flasks containing either 1 l of calcium sulfate or copper sulfate. The mixes were harvested after contact times (0.25, 0.5, 1.0, 2.0, and 4.0 hours) for determining total and calcium hardness levels and copper concentrations. Total hardness levels were quantified using the titrimetric method against ethylene diamine tetra-acetic acid; EDTA (0.01 mol.l−1) with aerochrom black-T indicator (American Public Health Association, 2012), calcium hardness levels were quantified using titrimetric method against EDTA with murexide powder indicator (American Public Health Association, 2012), and copper concentrations were quantified using atomic absorption spectrophotometer (American Public Health Association, 2012). The chelating efficacies (%) of *E. crassipes* 1% were calculated by proportionating the final to the initial concentrations.

In-vivo effectiveness of E. crassipes

Experimental birds' housing and biosecurity

We used 200 1-day-old Ross®308 broiler chicks from El-Helal Company—Egypt. We weighed the chicks on their arrival (W_0) and randomly divided them into 4 groups (50 birds each, 5 replicates of 10 birds) into units with a hay-deep litter system after Soliman and Hassan (2020b). The units were optimized with biosecurity measures following Soliman and Abdallah (2020a). These measures were foot dips of crude carbolic acid 5%, fly-proof nets, wild bird-proof entrances, secured food storage areas, proper interior arrangements to facilitate daily observation; feeding; and watering act, wire baited traps to discourage rodents, dry cleaning using brooms, wet cleaning using glutaraldehyde, disinfection using povidone-iodine 7.5%; sodium hydroxide 5%; and formaldehyde spraying respectively. The units were supplied after Soliman and Hassan (2019) with a continuous lighting program (23 hours L and 1 hour D) using a white light-emitting diode of 20 W. The ventilation act was served by negative cross-ventilation using side-wall V-shaped windows (air inlets), ceiling fans for proper air distribution, and side-wall suction fans across the rooms (air outlets). The units' floors were covered with a thin film of a chemical litter treatment (superphosphate) at a rate of 0.5 $g.m^{-2}$ after Soliman *et al*. (2018) to maintain litter abiotic conditions.

Experimental birds' microclimate and management

The room's microclimatic temperature was optimized at 35°C by turning on indirect heat sources (halogen and oil heaters) following Soliman *et al*. (2021a). This temperature was sufficient for brooding. Starting from the eighth day of the rearing trial, the temperature was reduced by 0.5°C daily (3.5°C/week) until achieving 25°C–28°C/third week of the trial. Chicks were given *ad libitum* access to the experimented treated or non-treated drinking water. The dietary requirements of the broilers were met by corn-soybean ration (El-Eman company, Egypt) following the National Research Council (1994), and Applegate and Angel (2014) modifications. The nutritive ingredients of the used starter (1–14 days) and the grower (15–38 days) rations were protein (22.5% and 21%), energy (2,930 and 3,150 kcal), crude fiber (3.79% and 3.35%), and fat (5.4% and 2.67%), respectively.

Broilers were massively immunized via dechlorinated drinking water vaccination act that was served after 2–4 hours of water deprivation. Birds were vaccinated against infectious bronchitis (7th day), infectious bursal disease (14th and 21st days), and Newcastle virus disease (16th and 26th days). Close observation was followed for the early detection of any abnormalities and mortalities. The experiment was designed to last for 38 days during which the daily prevailing microclimatic conditions were recorded.

***Eichhornia crassipes* treated water and challenges act**

Broilers were supplied daily with *E. crassipes* (1 $g.l^{-1}$) treated water for 4–6 hours, starting from the third-day-old throughout the experiment (38 days). Meanwhile, polluted water was supplied to groups as follows: G1 with calcium sulfate (75 $mg.l^{-1}$) polluted water, G2 with copper sulfate (200 $mg.l^{-1}$) polluted water, G3 with *S.* Typhimurium (1.5×10^{6} CFU.ml^{-1}) contaminated water, and G4 with non-polluted tap water (control negative). Polluted/contaminated water was supplied to birds weekly on the 7th, 14th, 21st, 28th, and 35th days. The repetition of the challenge was designed to evaluate the effectiveness of the *E. crassipes* to neutralize the impact of overwhelming challenges in broiler farms.

On the challenge day, water deprivation was run for 2–4 hours to ensure the weekly challenge act, and the *E. crassipes* treated water was supplied following the challenge.

Performance indices (PIs)

Representative simple random samples were harvested from broilers' groups (44 birds/ group) on their arrival and weekly for weighing the live body weight (LBW. g^{-1}) with a digital scale (WONHENG®, China). We calculated the sample size at a 95% confidence level following Thrusfield and Christley (2018) as follows:

$$n = (Z_{1-\alpha/2})^2.p\ (1-p)/d^2$$

where (n) is the samples' number, ($Z_{1-\alpha/2}$) is the normal standard, (p) is the probable population proportions, and (d) is the absolute precision.

The PIs were calculated following Soliman and Hassan (2017). The weight gains (WG.g^{-1}) are the differences between week-ending weights (Wx) to initial weights (W_0). Feed (FI.g^{-1}) and water (WI.ml^{-1}) intakes are the proportions between the amounts consumed in a group to the number of surviving birds in such a group. The feed conversion ratios (FCR) are the proportions of FI.g^{-1} to WG.g^{-1}, while the PI is the proportion of LBW. kg^{-1} to FCR.

Sampling

We collected 1,914 samples divided into 570 including 90 *E. crassipes* pollutants mixes (3 replicates × triplets × 2 pollutants × 5 sampling times) and 480 *E. crassipes* microbial mixes (96 well × 5 sampling times) from the *in-vitro* study, and 1,344 including 192 sera, 192 duodenal swabs, and 960 tissues (breast muscles, liver, spleen, heart, and bursa of Fabricius) from 5 bird groups after the *in-vivo* study. Nine ml buffered peptone water (Oxoid™) were used as a vehicle for the duodenal swabs.

We sacrificed 192 broilers (48/group) by the end of the clinical trial (38th day) via slaughtering with decapitation for harvesting blood samples on serum tubes (BD Vacutainer®). Samples were warmed in a water bath (Thermo®, Germany) at 28°C/20 minutes and centrifuged (Fisher® CL10) at 3,500 rpm/15 minute. Sera were pipetted (Thermo Scientific™) into Eppendorfs (2.5 ml) and stored at −20°C (Soliman *et al*., 2017). The carcasses were weighed (CW.g^{-1}) after de-feathering and evisceration. Breast muscles were incised for the bacteriological assessment. Edible organs (liver and heart) and immune organs (spleen, and bursa of Fabricius) were harvested and weighed (g). Carcasses were disposed of by burial with lime.

Biochemical profile and immunoglobulin concentrations

We analyzed sera (n = 192) calorimetrically using ROCHE® COBAS Integra 400 Plus chemical analyzer for some biochemical parameters [total protein (TP); g. dl^{-1}, alanine aminotransferase (ALT); $IU.l^{-1}$, urea; $mg.dl^{-1}$, creatinine (CREAT); $mg.dl^{-1}$, Glucose (GLUCO); $mg.dl^{-1}$, total cholesterol (TC); $mg.dl^{-1}$, and triglycerides (TGs); $mg.dl^{-1}$] and some antioxidant enzymatic activity [total antioxidant capacity (TAC); $mM.ml^{-}1$, malondialdehyde (MDA); $nmol.ml^{-}1$, and superoxide dismutase (SOD); $U.ml^{-}1$]. We analyzed sera also using ROCHE® ELECSYS 1010 Immunoassay Analyzer for hormonal and immunological profiles (Cortisol hormone; $mcg.dl^{-1}$ and immunoglobulin G and M; $mg.dl^{-1}$).

Bacteriological examinations

Frozen breast muscle samples (n = 192) were thawed, smashed in the stomacher (Seward®, UK), and added to 9 ml of buffered peptone water. Breast muscles (n = 192) and duodenal swabs (n = 192) solutions were diluted up to (10^{-8}) after American Public Health Association (2017) to cover the contamination range. We analyzed the dilutions for aerobic total bacterial (TBC) onto standard plate count agar (Oxoid™), Enterobacteriaceae (TEC) onto eosin methylene blue agar, and *Salmonella* counts (TSC) onto CHROMagar™ *Salmonella* (BD BBL*ei*) using the drop plate (Kim and Lee, 2016) at 37°C/24–48 hours. Ideal colonies were counted on the Dark-field colony counter (Murray *et al*., 2015).

Statistical analysis

We analyzed the data using the Statistical Package for Social Sciences (SPSS version 27.0, IBM Corp, NY, USA) software package (SPSS, 2020). The multifactorial two-way analysis of variance was employed to determine the overall influence of the variables (water pollutants and broiler's age) and their interactions. The statistical model is as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \mathcal{E}_{ijk}$$

where Y_{ijk} is the final measurement of the dependent variables; μ is the overall mean; α_i is the fixed effect of the pollutants, β_j is the fixed effect of the broiler's age, $(\alpha\beta)_{ij}$ is the interactions, and $\mathcal{E}_{ijk}$ is the random error. We expressed microbial counts as logarithms (Log) using Microsoft Excel 2016. We expressed the results as highly significant at ($p < 0.01$), significant at ($p \leq 0.05$), and non-significant at ($p > 0.05$).

Ethical approval

The laboratory and field clinical trials' designs and materials were approved by the Scientific Research Ethics Committee, Faculty of Veterinary Medicine, Suez Canal University–Ismailia, Egypt with approval number (2022025). Broilers were housed and approached in comfortable microclimatic conditions as much as possible to prevent the development of any overwhelming challenges other than the induced (Polluted and/or contaminated water). We minimized the number of birds as an understudy as possible and followed the laboratory animal rights (3Rs).

Results

Water analysis

Eichhornia crassipes treated water in Table 1 revealed highly significant ($p < 0.01$) decreases in

Table 1. Water analysis (Mean ± SE) in tap and *E. crassipes* treated water.

Elements	Tap water	Treated water	*p*-value	MPL
Physicochemical analysis				
pH	$7.20^{a} \pm 0.086$	$7.25^{a} \pm 0.066$	0.619	6.5–8.0
EC (µS.cm^{-1})	$574.6^{a} \pm 0.991$	$196.5^{b} \pm 0.429$	0.000	200
TDS (mg.l^{-1})	$385.0^{a} \pm 0.701$	$131.6^{b} \pm 0.328$	0.000	500
DO (mg.l^{-1})	$6.53^{b} \pm 0.169$	$7.93^{a} \pm 0.130$	0.001	10
T. alkalinity (mg.l^{-1})	$81.0^{a} \pm 0.769$	$34.1^{b} \pm 0.173$	0.000	120
T. hardness (mg.l^{-1} CaCO3)	$73.3^{a} \pm 0.204$	$27.2^{b} \pm 0.239$	0.000	500
PO4 (mg.l^{-1})	$0.00^{a} \pm 0.000$	$0.00^{a} \pm 0.000$	--	0.1
SO4 (mg.l^{-1})	$54.3^{a} \pm 0.682$	$16.7^{b} \pm 0.164$	0.000	250
NO3 (mg.l^{-1})	$8.00^{a} \pm 0.577$	$1.05^{b} \pm 0.144$	0.000	45
Electrolytes analysis				
Na+ (mg.l^{-1})	$123.3^{a} \pm 0.409$	$90.4^{b} \pm 0.383$	0.001	200
K+ (mg.l^{-1})	$11.2^{a} \pm 0.259$	$3.6^{b} \pm 0.346$	0.000	10
Ca2+ (mg.l^{-1})	$55.6^{a} \pm 0.587$	$39.7^{b} \pm 0.766$	0.003	75
Mg2+ (mg.l^{-1})	$22.6^{a} \pm 0.315$	$11.5^{b} \pm 0.765$	0.000	50
Cl− (mg.l^{-1})	$120.0^{a} \pm 0.802$	$99.6^{b} \pm 0.527$	0.000	200
Heavy metals analysis				
Fe2+ (mg.l^{-1})	$0.06^{a} \pm 0.001$	$0.02^{b} \pm 0.004$	0.063	0.0
Cu2+ (mg.l^{-1})	$0.06^{a} \pm 0.001$	$0.00^{b} \pm 0.008$	0.007	1.0
Zn2+ (mg.l^{-1})	$1.20^{a} \pm 0.002$	$0.25^{b} \pm 0.002$	0.000	5.0
Pb2+ (mg.l^{-1})	$0.00^{a} \pm 0.000$	$0.00^{a} \pm 0.000$	0.077	0.05
Microbial assessment				
TBC (CFU.ml^{-1})	$3.96^{a} \pm 0.053$	$2.28^{b} \pm 0.046$	0.000	Zero
TEC (CFU.ml^{-1})	$2.24^{a} \pm 0.080$	$0.65^{b} \pm 0.002$	0.000	Zero
TSC (CFU.ml^{-1})	$0.33^{a} \pm 0.001$	$0.00^{b} \pm 0.000$	0.005	Zero

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$). EC: Electrical conductivity as µS.cm^{-1}; TDS: Total dissolved solids as mg.l^{-1}; DO: Dissolved oxygen as mg.l^{-1}; T. alkalinity: Total alkalinity as mg.l^{-1}; T. hardness: Total hardness as mg.l^{-1} $CaCO_3$; PO_4: Phosphate as mg.l^{-1}; SO_4: Sulfate as mg.l^{-1}; NO_3: Nitrate as mg.l^{-1}; Na^+: Sodium as mg.l^{-1}; K^+: Potassium as mg.l^{-1}; Ca^{2+}: Calcium as mg.l^{-1}; Mg^{2+}: Magnesium as mg.l^{-1}; Cl^-: Chloride as mg.l^{-1}; Fe^{2+}: Iron as mg.l^{-1}; Cu^{2+}: Cobber as mg.l^{-1}; Zn^{2+}: Zinc as mg.l^{-1}; Pb^{2+}: Lead as mg.l^{-1}; TBC: Total bacterial logarithmic counts as CFU.ml^{-1}; TEC: Total Enterobacteriaceae logarithmic counts as CFU.ml^{-1}; TSC: Total *Salmonella* logarithmic counts as CFU.ml^{-1}; SE: Standard error.

physicochemical such as electrical conductivity (df = 17, F = 442.2, and p = 0.000), total dissolved solids (df = 17, F = 442.24, and p = 0.000), total alkalinity (df = 17, F = 66.9, and p = 0.000), sulfates (df = 17, F = 164.9, and p = 0.000), nitrates (df = 17, F = 136.1, and p = 0.000), and total hardness (df = 17, F = 138.4, and p = 0.000), electrolyte such as sodium (df = 17, F = 17.9, and p = 0.001), potassium (df = 17, F = 306.4, and p = 0.000), calcium (df = 17, F = 12.0, and p = 0.003), magnesium (df = 17, F = 20.7, and p = 0.000), and chloride (df = 17, F = 42.8, and p = 0.000), heavy metal such as iron (df = 17, F = 4.0, and p = 0.063), copper (df = 17, F = 9.3, and p = 0.007), and zinc (df = 17, F = 30.2, and p = 0.000), and microbial assessments such as aerobic TBC (df = 17, F = 552.3, and p = 0.000), TEC (df = 17, F = 110.5, and p = 0.000), and TSC (df = 17, F = 10.8, and p = 0.005) compared to tap water. *Eichhornia crassipes* treated water also revealed highly significant ($p < 0.01$) increases in the dissolved oxygen levels (df = 17, F = 43.0, and p = 0.000). pH levels reveal no-significant differences (df = 17, F = 0.258, and p = 0.619) in *E. crassipes* treated and tap water.

In-vitro adsorption and antimicrobial efficacies

Eichhornia crassipes 1% suspension reveals in Figure 1A 100% adsorption (chelating) activity ($p < 0.01$) for copper (Cu^{+2}, df = 44, F = 444.8, and p = 0.000) and total (df = 44, F = 6,124.5, and p = 0.000) and calcium (df = 44, F = 5,549.7, and p = 0.000) hardness after 1-hour. *Eichhornia crassipes* 1% suspension also reveals in Figure 1B 100% killing (antimicrobial) efficacy ($p < 0.01$) against *E. coli* O157: H7 (df = 119, F = 13,843.9, and p = 0.000), *S.* Typhimurium (df = 119, F = 2,595.2,

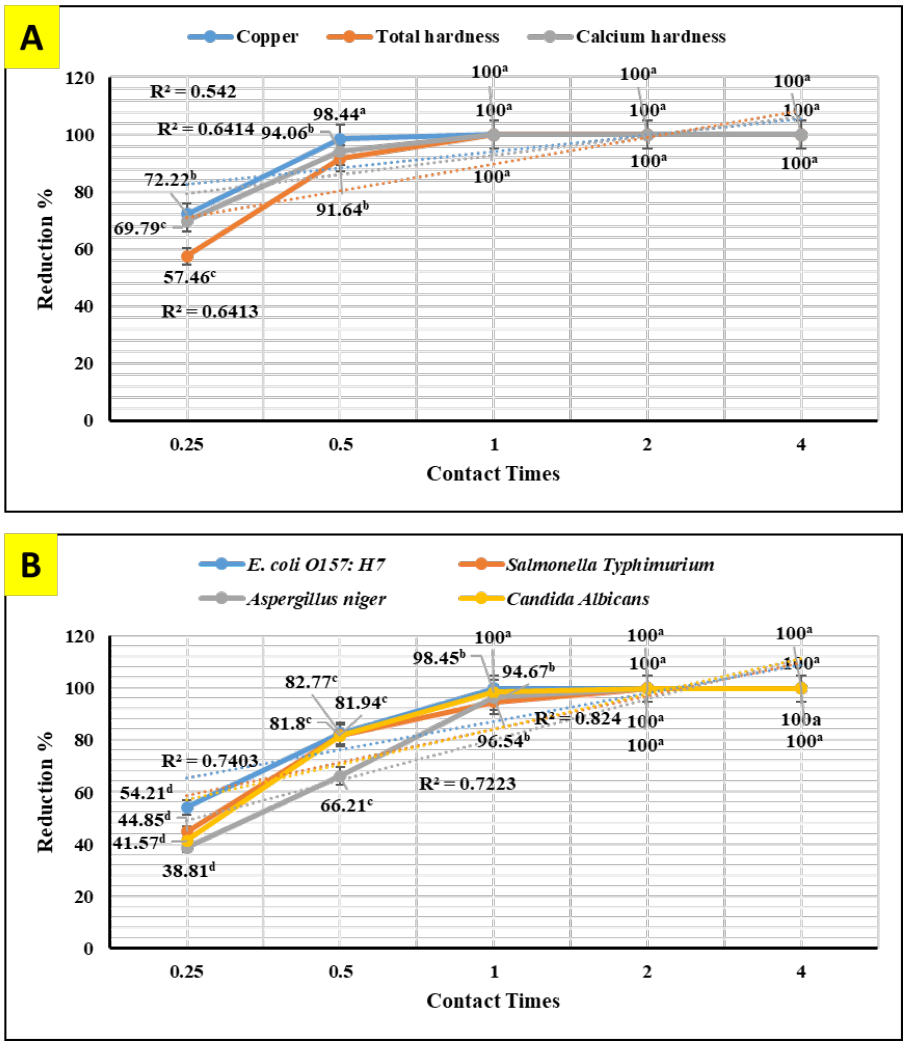

Fig. 1. *In-vitro* adsorption and antimicrobial efficacy (Reduction percentage Mean ± SE) of *E. crassipes* 1% suspension. (A) Chemical adsorption action. Initial levels of total hardness = 2,100 mg.l^{-1} $CaCO_3$, calcium hardness = 1,600 mg.l^{-1} $CaCO_3$, and copper = 5 mg/l. (B) Microbial survival. Initial levels of *E. coli* O157: H7 = 1.7 × 10^{11} CFU.ml^{-1}, *Salmonella* Typhimurium = 1.5 × 10^{6} CFU.ml^{-1}, *A. niger* = 2.5 × 10^{8} CFU.ml^{-1}, and *C. albicans* = 2.5 × 10^{8} CFU.ml^{-1}

and $p = 0.000$), *A. niger* (df = 119, $F = 7{,}738.1$, and $p = 0.000$), and *C. albicans* (df = 119, $F = 8{,}773.4$, and $p = 0.000$) after 1, 2, 2, and 2-hour respectively.

Growth traits

Eichhornia crassipes 1% treated water reveals in Table 2 highly significant ($p < 0.01$) improvements of WG (df = 3, $F = 11.1$, and $p = 0.000$), PI (df = 3, $F = 35.8$, and $p = 0.000$), WI interactions (df = 12, $F = 22.8$, and $p = 0.000$), WI/FI ratio (df = 3, $F = 48.9$, and $p = 0.000$), FCR (df = 3, $F = 24.5$, and $p = 0.000$), and FI (df = 3, $F = 47.1$, and $p = 0.000$) in all treated broiler groups compared to the control. The overall means concerning age reveal in Table 2 highly significant ($p < 0.01$) improvements of WG (df = 4, $F = 987.3$, and $p = 0.000$), FCR (df = 4, $F = 120.6$, and $p = 0.000$), and PI (df = 4, $F = 843.2$, and $p = 0.000$) in the fourth week, and WI (df = 4, $F = 1{,}021.8$, and $p = 0.000$) in the second week, as well as, highly significant ($p < 0.01$) decreases in FI (df = 4, $F = 4{,}914.4$, and $p = 0.000$) in the second week.

Live birds' weights and carcasses traits

Eichhornia crassipes 1% treated water reveals in Table 3 highly significant ($p < 0.01$) increases in LBW (df = 3, $F = 191.1$, and $p = 0.000$) in G3 (broilers challenged with *S.* Typhimurium) compared to other challenged groups and the control. Carcass weights (CW, Table 3) reveal highly significant ($p < 0.01$) increases (df = 3, $F = 256.2$, and $p = 0.000$) in G1 (broilers challenged with calcium sulfate) and G2 (broilers challenged with copper sulfate) with no significant differences between the two groups. Liver (df = 3, $F = 126.0$, and $p = 0.000$), heart (df = 3,

Table 2. PIs (Mean ± SE) in different groups supplied with *E. crassipes* treated water (1 g.l^{-1}).

Groups	Age W	WG g	FCR %	PI ratio	FI g	WI ml	WI/FI ratio
Overall means concerning treatments							
G1		$357^{a} \pm 0.056$	$1.3^{b} \pm 0.043$	$6.9^{a} \pm 0.069$	$472^{b} \pm 0.023$	$742^{ab} \pm 0.079$	$1.63^{ab} \pm 0.029$
G2		$352^{a} \pm 0.084$	$1.4^{b} \pm 0.065$	$6.8^{a} \pm 0.078$	$480^{b} \pm 0.034$	$740^{ab} \pm 0.092$	$1.59^{b} \pm 0.029$
G3		$358^{a} \pm 0.065$	$1.3^{b} \pm 0.062$	$7.1^{a} \pm 0.070$	$474^{b} \pm 0.029$	$756^{ab} \pm 0.088$	$1.65^{a} \pm 0.027$
Gc		$319^{b} \pm 0.042$	$1.7^{a} \pm 0.098$	$5.1^{b} \pm 0.004$	$520^{a} \pm 0.092$	$721^{b} \pm 0.076$	$1.38^{c} \pm 0.040$
p-value		0.014	0.006	0.002	0.001	0.154	0.007
Overall means concerning the broiler's age							
First week		$88^{e} \pm 0.022$	$1.2^{c} \pm 0.040$	$1.1^{e} \pm 0.004$	$106^{e} \pm 0.029$	$192^{e} \pm 0.023$	$1.80^{a} \pm 0.031$
Second week		$223^{d} \pm 0.017$	$1.6^{b} \pm 0.012$	$2.2^{d} \pm 0.003$	$356^{d} \pm 0.078$	$545^{d} \pm 0.076$	$1.52^{c} \pm 0.024$
Third week		$467^{b} \pm 0.076$	$1.2^{c} \pm 0.019$	$6.6^{c} \pm 0.007$	$589^{c} \pm 0.078$	$802^{c} \pm 0.073$	$1.37^{d} \pm 0.046$
Fourth week		$589^{a} \pm 0.012$	$1.0^{d} \pm 0.030$	$13.7^{a} \pm 0.004$	$617^{b} \pm 0.018$	$984^{b} \pm 0.020$	$1.59^{b} \pm 0.027$
Fifth week		$366^{c} \pm 0.011$	$2.1^{a} \pm 0.002$	$8.7^{b} \pm 0.003$	$764^{a} \pm 0.055$	$1,176^{a} \pm 0.067$	$1.53^{c} \pm 0.026$
p-value		0.000	0.001	0.000	0.000	0.000	0.002
Interactions between treatments by broiler's age							
G1	First week	$95^{e} \pm 0.020$	$1.1^{d} \pm 0.012$	$1.3^{e} \pm 0.023$	$105^{e} \pm 0.063$	$202^{e} \pm 0.014$	$1.93^{a} \pm 0.014$
	Second week	229d ± 0.217	$1.5^{b} \pm 0.014$	$2.4^{d} \pm 0.031$	$359^{d} \pm 0.022$	$557^{d} \pm 0.095$	$1.55^{b} \pm 0.047$
	Third week	$436^{c} \pm 0.111$	$1.2^{c} \pm 0.028$	$6.3^{c} \pm 0.024$	$561^{c} \pm 0.048$	$885^{c} \pm 0.072$	$1.57^{b} \pm 0.012$
	Fourth week	$599^{a} \pm 0.150$	$1.0^{d} \pm 0.025$	$13.8^{a} \pm 0.037$	$612^{b} \pm 0.014$	$965^{b} \pm 0.067$	$1.57^{b} \pm 0.054$
	Fifth week	$426^{b} \pm 0.186$	$1.7^{a} \pm 0.091$	$10.8^{b} \pm 0.054$	$724^{a} \pm 0.067$	$1,104^{a} \pm 0.027$	$1.52^{b} \pm 0.063$
G2	First week	$94^{e} \pm 0.153$	$1.1^{d} \pm 0.017$	$1.2^{e} \pm 0.035$	$107^{e} \pm 0.042$	$201^{e} \pm 0.095$	$1.87^{a} \pm 0.011$
	Second week	$221^{d} \pm 0.258$	$1.6^{b} \pm 0.020$	$2.2^{d} \pm 0.039$	$355^{d} \pm 0.099$	$538^{d} \pm 0.029$	$1.51^{c} \pm 0.063$
	Third week	$432^{b} \pm 0.211$	$1.3^{c} \pm 0.011$	$5.8^{c} \pm 0.065$	$587^{c} \pm 0.080$	$887^{c} \pm 0.093$	$1.51^{c} \pm 0.013$
	Fourth week	$678^{a} \pm 0.534$	$0.9^{e} \pm 0.050$	$16.3^{a} \pm 0.065$	$624^{b} \pm 0.056$	$978^{b} \pm 0.062$	$1.58^{b} \pm 0.065$
	Fifth week	$337^{c} \pm 0.128$	$2.1^{a} \pm 0.077$	$8.4^{b} \pm 0.038$	$726^{a} \pm 0.024$	$1,096^{a} \pm 0.068$	$1.51^{c} \pm 0.053$
G3	First week	$97^{e} \pm 0.214$	$1.1^{c} \pm 0.024$	$1.3^{d} \pm 0.049$	$107^{e} \pm 0.052$	$208^{e} \pm 0.069$	$1.94^{a} \pm 0.010$
	Second week	$226^{d} \pm 0.532$	$1.5^{b} \pm 0.037$	$2.3^{c} \pm 0.079$	$358^{d} \pm 0.054$	$588^{d} \pm 0.094$	$1.64^{b} \pm 0.027$
	Third week	$531^{b} \pm 0.134$	$1.1^{c} \pm 0.027$	$8.2^{b} \pm 0.029$	$586^{c} \pm 0.041$	$892^{c} \pm 0.087$	$1.52^{c} \pm 0.010$
	Fourth week	$580^{a} \pm 0.175$	$1.0^{c} \pm 0.032$	$14.5^{a} \pm 0.051$	$591^{b} \pm 0.033$	$990^{b} \pm 0.076$	$1.67^{b} \pm 0.054$
	Fifth week	$358^{c} \pm 0.178$	$2.0^{a} \pm 0.003$	$9.0^{b} \pm 0.049$	$729^{a} \pm 0.057$	$1,105^{a} \pm 0.098$	$1.51^{c} \pm 0.044$

Continued

Groups	Age W	WG g	FCR %	PI ratio	FI g	WI ml	WI/FI ratio
Gc	First week	$65^{e} \pm 0.228$	$1.6^{b} \pm 0.057$	$0.7^{d} \pm 0.003$	$106^{e} \pm 0.046$	$158^{e} \pm 0.084$	$1.48^{b} \pm 0.020$
	Second week	$216^{d} \pm 0.178$	$1.6^{b} \pm 0.016$	$2.0^{c} \pm 0.030$	$353^{d} \pm 0.037$	$497^{d} \pm 0.018$	$1.40^{c} \pm 0.015$
	Third week	$469^{b} \pm 0.312$	$1.3^{c} \pm 0.009$	$6.0^{b} \pm 0.006$	$624^{c} \pm 0.058$	$547^{c} \pm 0.026$	$0.87^{d} \pm 0.04$
	Fourth week	$500^{a} \pm 0.211$	$1.3^{c} \pm 0.055$	$10.2^{a} \pm 0.058$	$640^{b} \pm 0.063$	$1,003^{b} \pm 0.032$	$1.56^{a} \pm 0.038$
	Fifth week	$343^{c} \pm 0.307$	$2.7^{a} \pm 0.001$	$6.5^{b} \pm 0.045$	$877^{a} \pm 0.025$	$1,402^{a} \pm 0.059$	$1.59^{a} \pm 0.052$
p-value		0.001	0.002	0.001	0.000	0.002	0.001

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$). G1: Broilers challenged with calcium sulfate (75 mg.l^{-1}); G2: Broilers challenged with copper sulfate (200 mg.l^{-1}); G3: Broilers challenged *S.* Typhimurium (1.5×10^{6} CFU.ml^{-1}); G4: Control negative broilers; WG: Weight gain; FCR: Feed conversion ratio; PI: Performance index; FI: Feed intake; WI: Water intake; WI/FI: Water to feed intake ratio; SE: Standard error.

$F = 89.8$, and $p = 0.000$), and spleen (df = 3, $F = 355.3$, and $p = 0.000$) weights reveal highly significant ($p < 0.01$) increases in G3 compared to all other groups (Table 3). Bursa's weights (Table 3) reveal highly significant ($p < 0.01$) increases (df = 3, $F = 348.0$, and $p = 0.000$) in G3 and G2 with no significant differences between the two groups.

Biochemical, immunological, and hormonal profiles

Eichhornia crassipes 1% treated water reveals highly significant ($p < 0.01$, Table 4) improvements of the measured biochemical such as TP (df = 3, $F = 60.6$, and $p = 0.000$), ALT (df = 3, $F = 4,329.5$, and $p = 0.000$), urea (df = 3, $F = 142.4$, and $p = 0.000$), CREAT (df = 3, $F = 762.1$, and $p = 0.000$), GLUCO (df = 3, $F = 1,197.9$, and $p = 0.000$), TG (df = 3, $F = 17,063.2$, and $p = 0.000$), and TC (df = 3, $F = 557.1$, and $p = 0.000$), and antioxidant parameters such as TAC (df = 3, $F = 4,491.8$, and $p = 0.000$), MDA (df = 3, $F = 1,625.8$, and $p = 0.000$), and SOD (df = 3, $F = 640.0$, and $p = 0.000$) in all treated broiler groups compared to the control. We reported that *E. crassipes* 1% treated water reveals in Table 5 highly significant ($p < 0.01$, Table 5) increases in immunoglobulins G (df = 3, $F = 112.5$, and $p = 0.000$) and M (df = 3, $F = 36.3$, and $p = 0.000$) of G3 compared to the other treated groups and the control. Meanwhile, the cortisol hormone (Table 5) reveals highly significant ($p < 0.01$) decreases (df = 3, $F = 358.0$, and $p = 0.000$) in all treated broiler groups compared to the control.

Bacteriological assessments

Eichhornia crassipes 1% treated water in Figure 2 reveals highly significant ($p < 0.01$) decreases in the TBC of intestinal swabs (df = 3, $F = 29.9$, and $p = 0.000$) and breast muscles (df = 3, $F = 13.2$, and $p = 0.000$), TEC of intestinal swabs (df = 3, $F = 24.0$, and $p = 0.000$) and breast muscles (df = 3, $F = 7.6$, and $p = 0.000$), and TSC (duodenal swabs) (df = 3, $F = 5.0$, and $p = 0.002$) in all treated broiler groups compared to the control.

Discussion

Water quality in broiler farms contributes to the general conditions of production, performance, and reproduction (Maharjan *et al*., 2016). Broilers hold an important position among food animals, thus requiring a healthy environment and water of high quality to contribute to good health and production (Magor *et al*., 2013). Drinking water in broiler farms can be easily polluted/contaminated with a variety of micro-organisms through the birds themselves or the surrounding sources contributing to a potential risk to the broilers and humans (Van der *et al*., 2016). According to Maes *et al*. (2019), the arriving pollutants concerning some micro-organisms such as *Stenotrophomonas maltophilia*, *Pseudomonas geniculata*, and *Pseudomonas aeruginosa* contribute to a lowering in the water quality and characterization.

Table 3. Live, carcass, and organ weights (Mean ± SE) in different groups supplied with *E. crassipes* (1 g.l^{-1}) treated water.

Groups	LBW g	CW g	Edible organs weights g		Immune organs weights g	
			Liver	Heart	Spleen	Bursa of Fabricius
Overall means concerning treatments						
G1	1,836^{ab} ± 0.049	1,516^{a} ± 0.049	40.6^{c} ± 0.034	1.61^{b} ± 0.001	10.0^{b} ± 0.007	0.98^{b} ± 0.001
G2	1,817^{b} ± 0.032	1,507^{a} ± 0.032	42.3^{b} ± 0.030	1.60^{b} ± 0.002	10.0^{b} ± 0.002	1.05^{a} ± 0.004
G3	1,843^{a} ± 0.076	1,483^{b} ± 0.076	44.9^{a} ± 0.030	1.68^{a} ± 0.001	10.7^{a} ± 0.006	1.03^{a} ± 0.001
Gc	1,640^{c} ± 0.029	1,280^{c} ± 0.012	35.6^{d} ± 0.025	1.03^{c} ± 0.005	8.7^{c} ± 0.008	0.60^{c} ± 0.001
p-value	0.008	0.009	0.001	0.002	0.002	0.001

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$). G1: Broilers challenged with calcium sulfate (75 mg.l^{-1}); G2: Broilers challenged with copper sulfate (200 mg.l^{-1}); G3: Broilers challenged *S.* Typhimurium (1.5 × 10^{6} CFU.ml^{-1}); G4: Control negative broilers; LBW: Live body weight; CW: Carcass weight; SE: Standard error.

Table 4. Biochemical profile (Mean ± SE) in different groups supplied with *E. crassipes* (1 g.l^{-1}) treated water.

Groups	TP g.dl^{-1}	ALT IU.l^{-1}	UREA mg.dl^{-1}	CREAT mg.dl^{-1}	GLUCO mg.dl^{-1}	TG mg.dl^{-1}	TC mg.dl^{-1}
Overall means concerning treatments							
G1	5.9^{d} ± 0.006	3.4^{d} ± 0.003	19.6^{d} ± 0.003	0.1^{d} ± 0.001	66^{c} ± 0.005	59^{d} ± 0.001	93^{c} ± 0.003
G2	6.2^{c} ± 0.003	3.6^{c} ± 0.001	21.8^{c} ± 0.004	0.3^{c} ± 0.002	72^{b} ± 0.006	63^{c} ± 0.008	109^{b} ± 0.005
G3	6.6^{b} ± 0.007	3.7^{b} ± 0.004	25.4^{b} ± 0.002	0.4^{b} ± 0.001	74^{b} ± 0.004	67^{b} ± 0.002	111^{b} ± 0.009
Gc	6.9^{a} ± 0.005	9.7^{a} ± 0.007	29.3^{a} ± 0.004	0.9^{a} ± 0.003	104^{a} ± 0.003	97^{a} ± 0.005	162^{a} ± 0.007
p-value	0.001	0.000	0.000	0.001	0.004	0.000	0.002

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$). G1: Broilers challenged with calcium sulfate (75 mg.l^{-1}); G2: Broilers challenged with copper sulfate (200 mg.l^{-1}); G3: Broilers challenged *S.* Typhimurium (1.5 × 10^{6} CFU.ml^{-1}); G4: Control negative broilers; TP: Total protein; ALT: Alanine aminotransferase; UREA: Urea; CREAT: Creatinine; GLUCO: Glucose; TG: Triglycerides; TC: Total cholesterol; SE: Standard error.

Table 5. Immunoglobulin concentrations and stress markers (Mean ± SE) in different groups supplied with *E. crassipes* (1 g.l^{-1}) treated water.

Groups	Immunoglobulin concentrations		Stress marker			
	IgG mg.dl^{-1}	IgM mg.dl^{-1}	Cortisol mcg. dl^{-1}	TAC mM.l^{-1}	MDA nmol.ml^{-1}	SOD U.ml^{-1}
Overall means concerning treatments						
G1	1,546^{b} ± 0.638	438^{c} ± 0.917	6.9^{b} ± 0.007	2.34^{a} ± 0.005	36.8^{a} ± 0.001	311^{a} ± 0.028
G2	1,568^{b} ± 0.267	497^{b} ± 0.697	6.2^{c} ± 0.009	2.24^{c} ± 0.006	36.4^{b} ± 0.006	310^{a} ± 0.039
G3	1,685^{a} ± 0.765	542^{a} ± 0.720	5.7^{c} ± 0.002	2.27^{b} ± 0.003	37.0^{a} ± 0.006	306^{a} ± 0.201
Gc	1,264^{c} ± 0.644	321^{d} ± 0.632	15.3^{a} ± 0.007	1.24^{d} ± 0.005	23.4^{c} ± 0.007	166^{b} ± 0.065
p-value	0.003	0.001	0.001	0.000	0.001	0.004

Means carrying different superscripts in the same column are significantly different at ($p \leq 0.05$) or highly significantly different at ($p < 0.01$). Means carrying the same superscripts in the same column are non-significantly different at ($p < 0.05$). G1: Broilers challenged with calcium sulfate (75 mg.l^{-1}); G2: Broilers challenged with copper sulfate (200 mg.l^{-1}); G3: Broilers challenged *S. Typhimurium* (1.5 × 10^{6} CFU.ml^{-1}); G4: Control negative broilers; IgG: Immunoglobulin G; IgM: Immunoglobulin M; CORT: Cortisol; TAC: Total antioxidant capacity; MDA: Malondialdehyde; SOD: Superoxide dismutase; SE: Standard error.

Conventional water treatments (coagulation, sedimentation, flocculation, membrane filter, irradiation, and adsorption) influence the water's physicochemical characteristics as recorded by Robinson *et al*. (2001) to achieve water of good quality at a high cost, and the treatment process wastes require special treatment or disposal and thus increase the cost. On the other hand, Mishra and Maiti (2017) revealed

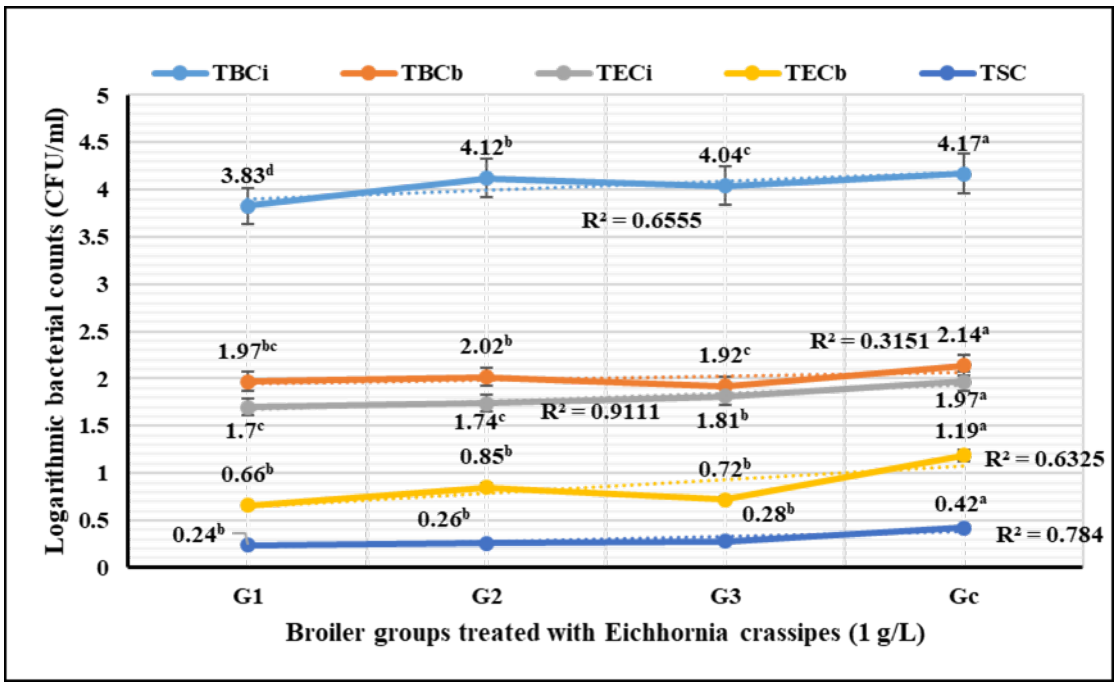

Fig. 2. Logarithmic bacterial counts (Mean) in different groups supplied with *E. crassipes* (1 g.l^{-1}) treated water. G1 = Broilers challenged with calcium sulfate (75 mg.l^{-1}), G2 = Broilers challenged with copper sulfate (200 mg.l^{-1}), G3 = Broilers challenged *Salmonella* Typhimurium (1.5 × 10^{6} CFU.ml^{-1}), G4 = control broilers. TBCi = Total bacterial counts in intestinal swabs, TBCm = Total bacterial counts in breast muscles, TECi = Total *Enterobacteriaceae* counts in intestinal swabs, TECm = Total *Enterobacteriaceae* counts in breast muscles, TSC = Total *Salmonella* count in intestinal swabs.

that the adsorption and phytoremediation processes are waste-free and provide higher advantageous water conditions compared to other treatments as they use natural and biological products such as rice husk, bark, coal, wood dust, tree bark powder, lignin, seaweeds, orange peel, banana peel, coconut pulp, and *E. crassipes*. *Eichhornia crassipes* is advantageous by higher adsorption capabilities in shorter time and accumulation (leaves, roots, and bulb) for long periods against chemical and biological pollutants (Thapa *et al*., 2016), and can be used in biogas production, feed product for fish and animals, microbial metabolism because of its high carbon contents, vermicomposting field, and in medicinal uses (Sharma *et al*., 2016).

Eichhornia crassipes reveals some capabilities in different studies such as enhancing the water quality characteristics, minimizing the pollutants' (heavy metals, and dyes) concentrations (Priya and Selvan, 2017), minimizing the biological oxygen demands (Wei *et al*., 2019), extract palm oil from mill waste in Malaysia, bio-indication for heavy metal pollution of rivers in Pakistan (Srivastava *et al*., 2019), Phyto-purification of wastewater (Victor *et al*., 2016), reduction of chromium levels (99.5%) in wastewater 15 days post-treatment (Saha et al., 2017), and extreme reduction of nitrates in wastewater combined with eutrophication (Wenwei *et al*., 2016).

Eichhornia crassipes treated water in the current study reveals highly significant declines in physicochemical, electrolytes, heavy metals, and microbial assessments and a highly significant increase in dissolved oxygen levels compared to tap water. The results agreed with those of Wuijts *et al*. (2018) who recorded that *E. crassipes* can actively minimize heavy metals and coloring agent concentrations from the water through bio-sorption, precipitation, and accumulation. Hartmann *et al*. (2018) also reported the high capabilities of *E. crassipes* to absorb dyes and heavy metals using electrostatic forces and improve water quality characteristics. Mahmood *et al*. (2010) recorded that *E. crassipes* water treatment contributed to a significant reduction of the biological and chemical oxygen demand (70.0%), the total solids (50.6%), and pH to a level that counteracts microbial survival. Also in agreement with our results, Dar *et al*. (2011) evaluated the capability of *E. crassipes* to treat different dilutions of wastewater (25%, 50%, 75%, and 100%) and recorded significant improvements in the physicochemical and biological parameters with significant increases of the dissolved oxygen levels. They recommended *E. crassipes* as an adsorbent to treat wastewater from agricultural, industrial, and municipal sources.

Eichhornia crassipes 1% suspension in the current results achieved 100% reductions of cobber and

total and calcium hardness after 1-hour and 100% antimicrobial efficacies against *E. coli* O157: H7, *S.* Typhimurium, *A. niger*, and *C. albicans* after 1, 2, 2, and 2-hours, respectively. The results were synchronized with those of Vishwakarma *et al*. (2018) who reported that *E. crassipes* contributed to a shift in the pH up to 8.6 which is unfavorable to microbial growth and survival, and a significant reduction in the levels of total dissolved solids, conductivity, total hardness, biological and chemical oxygen demands, nitrate-nitrogen, and ammonium nitrogen in the treated water. Our results were also synchronized with those of Long *et al*. (2018) who revealed the strong abilities of *E. crassipes* for minimizing copper, cadmium, lead, zinc, chromium, and iron up to 97.3%, 94.8%, 94.7%, 96.8%, 94.3%, and 93.5%, respectively. Deng *et al*. (2018) recorded in agreement with the current results the strong antimicrobial activity of *E. crassipes* against *Mycobacterium phlei*, *Candida parapsilosis*, and *Rhizopus oryzae*.

The field clinical trial reveals highly significant improvements in PIs, live weights, carcasses quality, biochemical parameters, immunoglobulin concentrations, cortisol hormone, and bacteriological assessments in broilers supplemented with *E. crassipes* treated water compared to the control group. The results agreed with those of Che *et al*. (2020) who recorded similar capabilities of *Eichhornia* purpurea (100, 200, and 600 mg.l^{-1}) treated water which enhanced weight acquires and carcasses yield and they recommended E. purpurea (200 mg.l^{-1}) as a natural effective eco-friendly water treatment. Abdel Shafy *et al*. (2016) reported that *E. crassipes* collected from the Nile River in Egypt contain some nutritive ingredients such as protein (10.7%), fat (4.94%), crude fiber (17.9%), nitrogen (44.3%), calcium (1.42%), and phosphorus (0.58%) making it suitable as a feed supplement with enhanced weight gains and improved feed conversions, and potential adsorbent of heavy metals polluting the drinking water.

Jianbo *et al*. (2008) found an increased performance, FI, and egg production in ducks supplied with *E. crassipes* treated wastewater, and they attributed these improvements to the higher crude protein contents of the supplied *E. crassipes*. Rufchaei *et al*. (2021) documented an improvement in the marketing weights, weight gains, and growth rates in fish fed with 1.5% of *E. crassipes* than those fed 0%, 0.5%, and 1% of *E. crassipes* leaves powder. Dumaup *et al*. (2020) from another perspective revealed no significant effect on the broiler's performance except for FI when supplied with *E. crassipes*. Abdulganiyu *et al*. (2013) also recorded no significant differences in initial and final body weights, FIs, and FCR among day-old Isa Brown pullets that received *E. crassipes* (0%, 5%, 10%, and 15%) for 8 weeks.

The high recorded improvements in broilers supplemented with *E. crassipes* treated water in our study might be attributed to the highly digestible protein contents. Wimalarathane and Perera (2019) recommended the use of *E. crassipes* as a dietary supplement in animals and poultry for its nutritive value and low cost. Indulekha *et al*. (2019**)** also recommended the use of *E. crassipes* as a dietary supplement to increase the production in animals and poultry. The recorded prominent immune-stimulant, antioxidant activities, and the biochemical enhancements of *E. crassipes* treated water in our study could be attributed to its chemical composition that exhibits anti-inflammatory, antifungal, antibacterial, and anticancer functions. The results were synchronized with those of Guna *et al*. (2017) who showed an increase in DNA inhibition and DPPH radical scavenging properties of *E. crassipes*.

Eichhornia crassipes in our study could act as a phytoremediation agent and sanitize water from all the chemical pollutants and microbial contaminants used (challenges) contributing to the production of high-quality water, enhanced production, performance, and immunity. The same concept was concluded by Maharjan (2016) who reported that water entering the broiler farms usually is contaminated with a microbial load of up to 300 CFU.ml^{-1}, but hence the breeders disinfect water using chemical or eco-friendly alternatives contributing to enhanced performance. Jacobs *et al*. (2020) also reported that sanitized and/or treated water enhanced significant growth traits.

Conclusion

Eichhornia crassipes 1% contributed to significant enhancements in the water's physicochemical characteristics such as declines of conductivity, total dissolved solids, total alkalinity, hardness, sulfates, nitrates, electrolyte (sodium, potassium, calcium, magnesium, and chloride), heavy metals (iron, copper, and zinc), and microbial counts (TBC, TEC, and TEC). *Eichhornia crassipes* 1% was also able to significantly increase the levels of dissolved oxygen. *Eichhornia crassipes* 1% suspension exhibited 100% adsorption activities for copper (5 mg.l^{-1}) and total (2,100 mg.l^{-1} $CaCO_3$) and calcium (1,600 mg.l^{-1} $CaCO_3$) hardness after 1-hour, and 100% antimicrobial activities against *E. coli* O157: H7 (1.7×10^{11} CFU.ml^{-1}), *S.* Typhimurium (1.5×10^{6} CFU.ml^{-1}), *A. niger* (2.5×10^{8} CFU.ml^{-1}), and *C. albicans* (2.5×10^{8} CFU.ml^{-1}) after 1, 2, 2, and 2-hours respectively.

The clinical trial showed that *E. crassipes* 1% suspension significantly improved PIs (LBW, WG, FI, WI, FCR, and PI), carcasses quality, biochemical parameters, antioxidant enzymatic activities, immunoglobulin concentrations, cortisol hormone, and intestinal microbiota in all treated broilers. Thus, *E. crassipes* 1% could be used with great potential as an eco-friendly alternative for the water and wastewater treatment processes to reduce waste and treatment costs and provide broilers with water of good quality.

Authors' contributions

ESS deliberated and executed the laboratory and field trails' design, run water physicochemical analysis, water, and broiler samples' microbial examination, participated in sera samples analysis for biochemical and antioxidant profiles, engaged in writing the manuscript, and reviewed the submitted version. RHA calculated and judged the growth traits, determined carcass characteristics, analyzed sera for biochemical and antioxidant profiles, and collaborated in the manuscript writing. DSF took a part in the water physicochemical analysis, biochemical analysis, and microbial assessments, and collaborated in the manuscript writing.

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Conflict of interest

The authors declare that they have no competing interests or personal relationships that could influence the work reported in this manuscript.

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