Research Paper

Effect of natural disinfectant (*Moringa oleifera*) and a chemical disinfectant (calcium hypochlorite) on nematode eggs: bioefficiency and impact of physico-chemical variables

Arnold Landry Fotseu Kouam and Gideon Aghaindum Ajeagah

ABSTRACT

The aim of this study is to determine the effectiveness of disinfectant on the viability of eggs from three nematode species (*Ascaris*, *Trichuris*, *Ankylostoma*). It was conducted in a microcosm from June 2018 to June 2019. The wastewater scan was sampled using 5 L sterile containers, the sample was arranged in four replicas, three tests and one control. The test samples received three disinfectants (*Moringa*, calcium hypochlorite and *Moringa* associated with calcium hypochlorite) at varying concentrations. The physical and chemical parameters were measured before and after the application of each disinfectant. The samples were then observed under an optical microscope. The viability of the eggs was determined by incubating the Petri dish samples at 30 °C for 30 days. The analyses show that some physicochemical parameters can significantly influence the efficacy of disinfectant on the eggs. The calcium hypochlorite associated with *Moringa* at 0.6 g/L showed greater efficacy on reducing viability and inactivation of eggs with 100% efficacy yield rates on *Ankylostoma* and *Trichuris trichiuria* and 97% on *Ascaris lumbricoides* eggs; this efficacy is significantly different from that observed on samples treated with *Moringa* and simple calcium hypochlorite. Of the three parasites tested, *A. lumbricoides* showed greater resistance to the disinfectant.

Key words | disinfectants, nematodes, sustainability, treatment wastewater

Arnold Landry Fotseu Kouam (corresponding author)
Gideon Aghaindum Ajeagah
Faculty of Sciences,
The University of Yaounde I,
Yaounde,
Cameroon
E-mail: *landryfotseu@gmail.com*

INTRODUCTION

Soil transmitted helminth infections (STH) are among the most common infections in low- and middle-income countries (WHO 2015). This infection remains of public health importance, particularly in developing countries (Nasution *et al.* 2019). The presence of parasitic helminths, particularly intestinal nematodes (*Ascaris*, *Trichuris*, *Ankylostoma*), is the main constraint for the reuse of wastewater because of their low infectious dose and long survival. Analysis of the health risks associated with these agents shows that these parasites differ markedly from bacteria and viruses in their resistance to the environment and by their low infectious dose (Eisenberg *et al.* 2002). According to the studies of Thompson (2004), helminth eggs can survive in the environment for several months. STH infections affect over 1.5 billion people worldwide (Dukpa *et al.* 2020). There are four important species of STHs that infect humans: *Ascaris lumbricoides* (round-worm), *Trichuris trichiura* (whip-worm) and *Ankylostoma*

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duodenale and *Necator americanus* (hookworms) (Bethony *et al.* 2006). Global estimates report 804 million people infected with roundworm, 477 million with whipworm and 472 million with hookworms (Jourdan *et al.* 2017).

The highest number of cases of helminthiasis in Africa (173 million) occurs in large numbers of children under the age of 15 years (Jimenez-Cisneros & Maya-Rendon 2007). According to Keffala *et al.* (2012), infection occurs as a result of ingestion of eggs from the soil or contaminated water (*A. lumbricoides* and *T. trichiuria*) or by active penetration of larvae through the skin (*Ankylostoma*). The viability of helminths in wastewater varies and depends on environmental conditions (Pecson *et al.* 2007). Thanks to their triple membrane, helminth eggs are considered to be the biological agents most resistant to aggression scans of the extra-intestinal environment (Wharton 1983). Due to its climate, Africa remains a very favourable territory for helminth resistance in wastewater. Studies carried out to date on helminths in wastewater have focused mainly on diagnostics (Ajeagah & Fotseu 2019) and the study of viability (Khallaayoune & Fatiha 1995; Keffala *et al.* 2012; Amoah *et al.* 2017) of helminths in wastewater. It is therefore essential to look more closely at the study of the resistance of nematodes to disinfectants and the abiotic factors that promote this resistance. The objective of this study is to assess the efficacy of *Moringa oleifera*, calcium hypochlorite and *M. oleifera* associated with calcium hypochlorite on the viability of Nematoda eggs (*A. lumbricoides*, *T. trichiura*, *Ankylostoma*) isolated from the wastewater of toilets of the University City of Yaounde University.

MATERIAL AND METHODS

Study frame and sampling station

This study took place from June 2018 to June 2019, and was carried out in two phases. The first phase of this three-month study (June to August) consisted of series of screening tests to determine ranges of minimum concentrations with observable effects on eggs. At this screening, two disinfectants (calcium hypochlorite, *Moringa*) and six concentration ranges were used for the analyses, namely, 0.1 g/L; 0.2 g/L; 0.3 g/L; 0.4 g/L; 0.5 g/L and 0.6 g/L.

Description of sampling station: University City effluent (CU)

The University City (CU) of the University of Yaounde I is the largest university city in Cameroon because of its capacity and is home to many students. This city generates a large capacity of wastewater, and this wastewater is connected to an underground channel that allows it to be transported to a treatment station; this station is currently non-functional and the wastewater ends up directly in the environment without treatment. The eggs of *Ascaris*, *Trichuris* and *Ankylostoma* for analysis were collected from the aeration channel of the wastewater.

Preparing disinfectant solutions

During this study we used three disinfectant solutions:

Solution 1: This solution was prepared from calcium hypochlorite ($CaCl_2$) crystals weighed with a scale at concentrations (0.1 g/L; 0.2 g/L; 0.3 g/L; 0.4 g/L; 0.5 g/L and 0.6 g/L).

Solution 2: This solution was prepared from water extracts from *M. oleifera* leaves weighed with a scale at concentrations (0.1 g/L; 0.2 g/L; 0.3 g/L; 0.4 g/L; 0.5 g/L and 0.6 g/L).

Solution 3: The third disinfectant solution was prepared from a mixture of equal proportions of calcium hypochlorite crystals (0.1 g/L; 0.2 g/L; 0.3 g/L; 0.4 g/L; 0.5 g/L and 0.6 g/L) and watery extracts from the leaves of *M. oleifera* concentrations (0.1 g/L; 0.2 g/L; 0.3 g/L; 0.4 g/L; 0.5 g/L and 0.6 g/L).

Sample for physicochemical and biological analyses

The wastewater samples were collected on the recommendations of Rodier (2009) for physical analysis and following the approach proposed by Keffala *et al.* (2012) for biological analysis. Samples were taken directly from the toilet effluent using a sterile 1 L bottle and taken back to the laboratory. The sedimentation technique was used to concentrate the eggs to facilitate their observation.

Measurement of physicochemical parameters

The physicochemical parameters assessed during this study were measured using conventional techniques described by Rodier (2009) with the appropriate reagents. The pH and electrical conductivity were measured using a portable multimeter. Ammonia nitrogen, nitrate, orthophosphates, suspended materials and turbidity were measured in the laboratory using a spectrophotometer. The oxidation was measured in the laboratory by volumetry.

Experimental device

We made a device comprising three sets (one series for each disinfectant) of 18 Erlenmeyer flasks (six Erlenmeyer flasks for each series), then we introduced 500 mL of sample into each Erlenmeyer, and each disinfectant was introduced into three Erlenmeyers at different concentrations (0.1 g/L; 0.2 g/L; 0.3 g/L; 0.4 g/L; 0.5 g/L and 0.6 g/L). The samples were then homogenized to allow perfect contact of the disinfectant and parasites. A contact time of 24 hours was observed for each sample. Five mL of the bottom of the sample was taken from each sample, then washed with sterile water and sodium thiosulfate twice to remove all the disinfectant in the sample (Stien 1989).

Parasite sustainability analysis

Viability tests of helminth eggs are done by colouring through specific dyes such as saffron, crystal violet, methylene blue, trypan blue, or by incubation. We used incubation tests to test the viability of nematodes. Five mL of the bottom of the sample was incubated on Petri dishes at 30 °C for 30 days (Massara *et al.* 2001; Pecson *et al.* 2007). After incubation the process of reducing the viability of the eggs was highlighted, through blastomere formation and embryo mobility. Then the eggs were examined under microscopy. In non-viable eggs segmentation stopped while viable eggs continued their segmentation and development until the formation of at least eight blastomeres for *Ascaris* eggs and *Ankylostoma* (Stien 1989; Keffala *et al.* 2012), by the mobility of the hexacanth embryo for *Trichuris* eggs, or by the ability to produce embryos (Hajjami *et al.* 2013). The identification was made through morphological analysis of the size shape and content of the eggs. Changes in blastomeres, oncosphere and hexacanthe embryos were highlighted under an optical microscope at 40× and 100×. Mensuration was made using an eye micrometer and the photos were taken using a Xploview model photographic device connected to one of the microscope eyes. The observations were repeated twice.

Statistical analysis

The normality of the data was assessed using the Kolmogorov–Smirnov test, while data comparisons were made using the analysis of variance (ANOVA) test and Student's *t*-test. The correlations were made using the Pearson test. All these analyses were carried out using SPSS version 17.0 software.

RESULTS

Morpho-pathological description of nematode eggs

In the course of this study, we found that the morphological and structural appearance of incubated eggs varies according to its species. The eggs of *Ankylostoma* obtained after incubation showed a succession of segmentation of the cytoplasm leading to blastomere formation after 4 weeks (Figure 1(a)). Conversely, in non-viable hookworm eggs, the egg segmentation process stopped after a few days, the cytoplasmic content of these eggs resulted in a simple inert cytoplasmic mass (Figure 1(b)). The viable *Ascaris* eggs obtained after incubation also resulted in the formation of more than eight blastomeres after 4 weeks of incubation (Figure 1(d)). However, non-viable *Ascaris* eggs were not segmented and the cytoplasmic content remained compact and inert (Figure 1(c)). In some *Ascaris* eggs, the outermost membrane had a slight rupture in irregularities. Viable *Trichuris* eggs obtained after application of disinfectants had a mobile hexacanthe embryo (Figure 1(f)), while the resulting non-viable *Trichuris* eggs had an immobile hexacanthe embryo (Figure 1(e)). Some of the non-viable eggs observed had a slight rupture of the cytoplasmic membrane at the junction between the mucous plugs and the rest of the cytoplasm.

Spatial variation in the number of viable eggs during treatment

Figure 2(a) shows spatial variations in the number of viable *A. lumbricoides* eggs counted during the study, before (control) and after application of each disinfectant at different

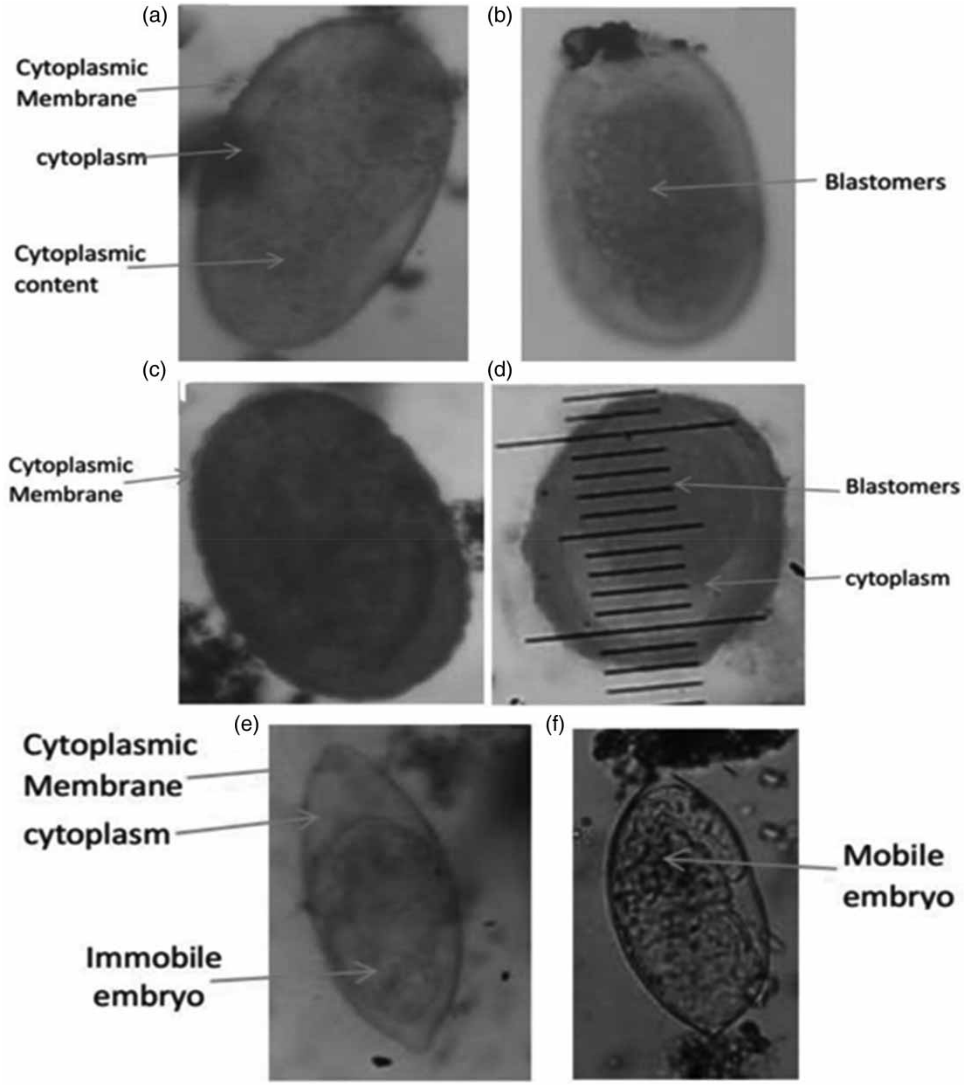

Figure 1 | Image of viable eggs (b), (d) and (f) and non-viable eggs (a), (c) and (e) obtained during the study; (a), (c) and (e) – unviable eggs of *Ascaris lumbricoides, Ankylostoma* and *Trichuris trichiura*, respectively; (b), (d) and (f) – viable eggs of *Ascaris lumbricoides, Ankylostoma* and *Trichuris trichiura*, respectively.

concentrations (C1 to C6). In total, 3,500 viable eggs were identified on the control sample. Overall, the values gradually drop on the samples treated with the different disinfectants depending on the concentrations used and the nature of the disinfectant. The lowest number of viable eggs (94 eggs/L) was obtained with *Moringa* associated with calcium hypochlorite at C6 concentration. There is a significant difference between the values obtained at C5 and C6 concentrations and the other concentrations. The values obtained from the control sample differ significantly ($p < 0.05$) from the values obtained with the other three disinfectants (Figure 2(b)).

Figure 2(c) shows the variation profile of the *Ankylostoma* eggs, with 2,060 viable eggs being counted on the control sample (before disinfection). After application of disinfectants the highest concentration was obtained with calcium hypochlorite at C1 concentration. The values obtained at C1 to C4 concentrations remain above 165 eggs/L. These values differ significantly from those (66 to 0 eggs/L) obtained at C5 and C6 concentrations. The lowest values were obtained with the *Moringa* associated with chloride (Figure 2(d)). The value of the control sample differs significantly from the values of the treated samples ($p < 0.05$).

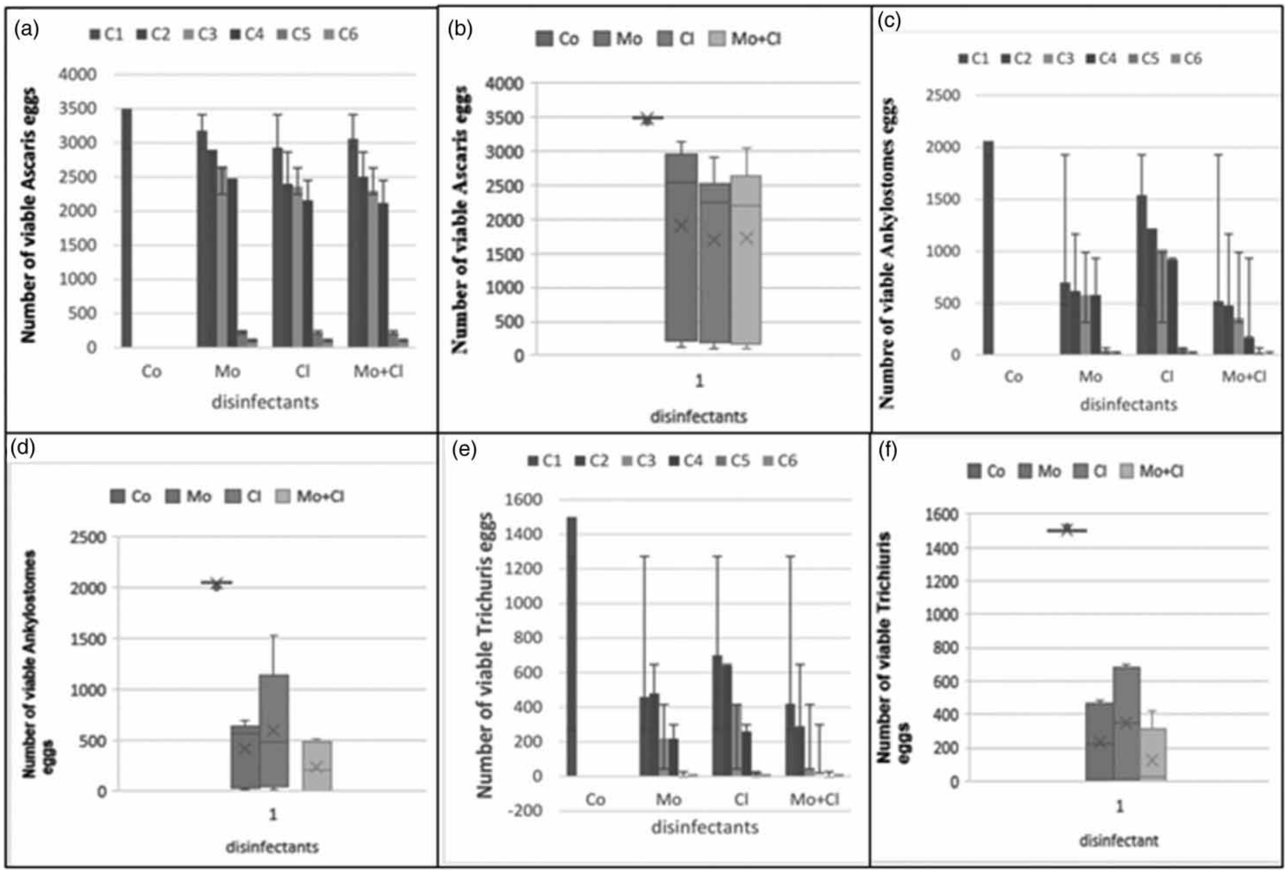

Figure 2 | Change in the number of eggs of *Ascaris*, *Ankylostoma* and *Trichuris* based on the different concentrations of disinfectants used. Co: control; Mo: *Moringa*; Cl: calcium hypochlorite; Mo-Cl: *Moringa* associated with chlorine.

Calcium hypochlorite is less effective as compared to the other two disinfectants (Mo and Mo + Cl).

The density of *T. trichiura* eggs varies significantly depending on the disinfectants used and the doses of concentrations applied. Densities of 1,500 eggs/L (Figure 2(e)) were observed on the control sample, but these densities dropped significantly on the treated samples. The highest value after treatment was obtained from the chlorine-treated sample at C1. A significant difference is noticeable from the C5 concentration where densities of 8 eggs/L, 28 eggs/L and 0 eggs/L were obtained for Mo, Cl and Mo + Cl, respectively. There is a significant difference between the control value and those obtained after the application of disinfectants ($p < 0.05$). Mo + Cl has the best results compared to the other two disinfectants (Figure 2(f)) from the C4 concentration and no viable eggs were detected on samples treated with Mo + Cl.

Figure 3 shows a grouping of helminth species undergoing treatment. The control sample shows an average of 2,300 eggs/L, but after disinfection these values drop below 1,500 eggs/L. The layout of the three graphs in Figure 3 shows that the values obtained with Mo + Cl are slightly lower than the values obtained with the other two disinfectants separately.

Temporal–spatial variation of physicochemical parameters

Ammoniacal nitrogen and nitrate

The value of the ammoniacal nitrogen obtained in the control sample before disinfection is 16 mg/L, and the application of the various disinfectants has reduced with the lowest levels of NH_4^+ obtained being 4 mg/L, 0.9 mg/L and 1.33 mg/L,

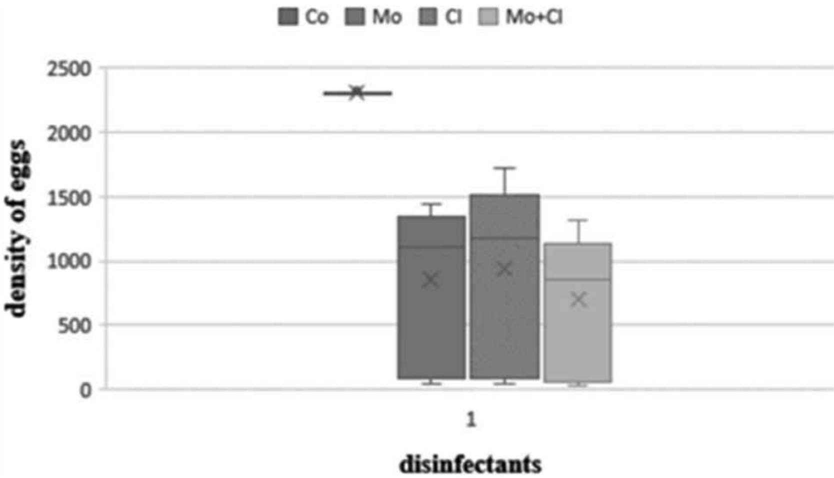

Figure 3 | Variations of microorganisms based on disinfectants.

respectively, for Mo, Cl and Mo + Cl (Figure 4(a)). Overall, calcium hypochlorite has lower values compared to the other two disinfectants. The nitrate variation profile is the same as ammoniacal nitrogen. The control value obtained before application of disinfectants is 151 mg/L and after disinfection the smallest values obtained are 32 mg/L, 10 mg/L and 19 mg/L, respectively, for Mo, Cl and Mo + Cl Figure 4(b)).

Orthophosphate and electrical conductivity

The orthophosphate content obtained in the control sample is 16 mg/L, and in the treated samples the values vary from 11 mg/L to 3 mg/L for *Moringa*, from 8 mg/L to 0.29 mg/L for calcium hypochlorite and 5.59 mg/L to 4.63 mg/L for *Moringa* associated with calcium hypochlorite (Figure 5(a)). There is no significant difference between orthophosphate levels obtained after disinfection. The value of electrical conductivity obtained in the control sample before disinfection is 6,805 μS/cm. After applying the various disinfectants, the lowest values of conductivity are 1,929 μS/cm, 797 μS/cm and 443 μS/cm, respectively, for MO, Cl and Mo + Cl (Figure 5(b)). These values differ significantly from those obtained before disinfection ($p < 0.05$).

Suspended solids and turbidity

The suspended solids levels obtained before disinfection are 1,645 mg/L, and after disinfection the lowest values

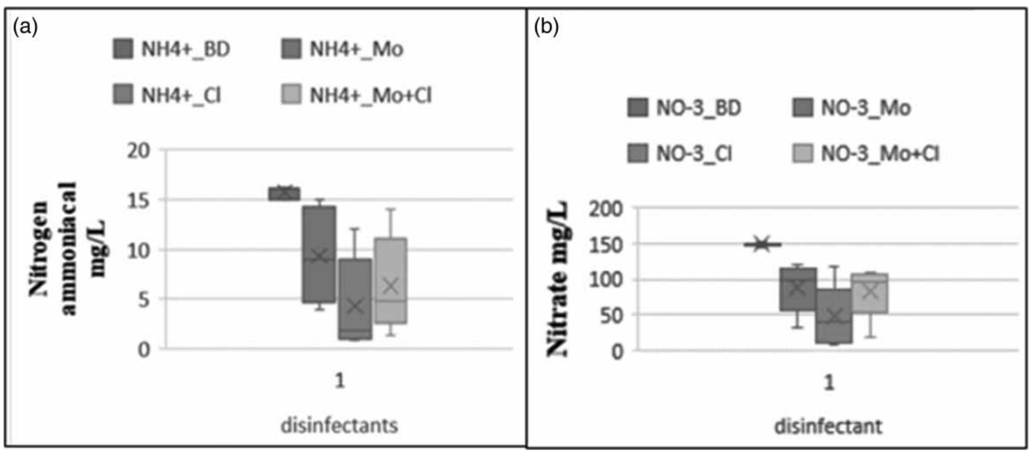

Figure 4 | Variation of ammoniacal nitrogen and nitrate levels based on disinfectants used.

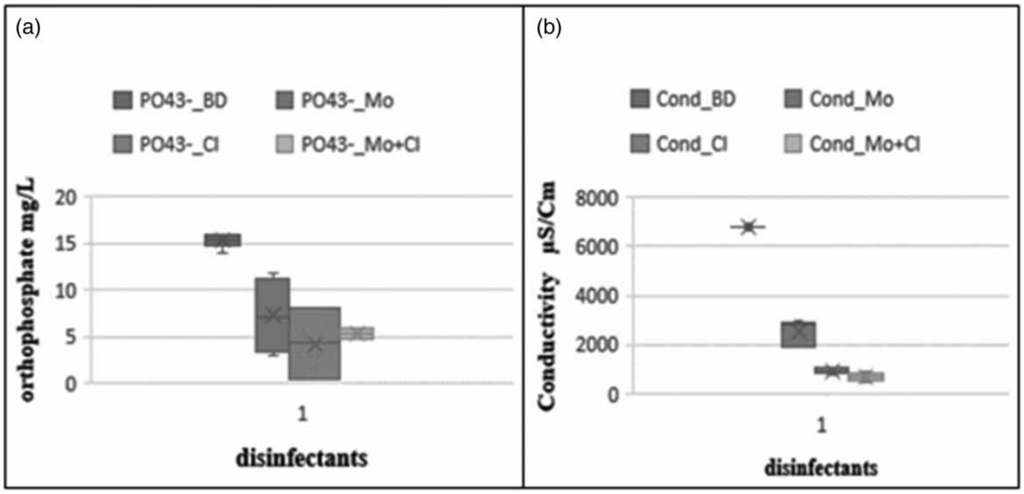

Figure 5 | Variation of orthophosphate (a) and electrical conductivity (b).

obtained are 640 mg/L, 180 mg/L and 220 mg/L, respectively, for Mo, Cl and Mo + Cl. The value of turbidity obtained before disinfection in the control sample is 1,645 FTU (Figure 6(a)), and the values obtained after disinfection are all below 120 FTU. The smallest values obtained are 25 FTU, 58 FTU and 8 FTU, respectively, for *Moringa*, Cl and Mo + Cl (Figure 6(b)). The values of suspended solids and turbidity obtained before disinfection differ significantly from those obtained after disinfection. For turbidity, Mo + Cl produced the lowest levels compared to the other two disinfectants, while the lowest suspended solids were obtained in the sample treated with calcium hypochlorite.

Oxidability and pH

The oxidative content obtained in the control sample is 14.81 mg/L, and after application of the different disinfectants the values vary from 8 mg/L to 5.13 mg/L for *Moringa*, 3 mg/L to 0 mg/L for calcium hypochlorite and 8 mg/L to 5.92 mg/L for *Moringa* combined with calcium hypochlorite (Figure 7(a)). Only calcium hypochlorite produced 0 mg/L. The value of pH obtained before disinfection in the control sample is levels (7.59). After application of the various disinfectants, the samples treated with simple chlorine have slightly basic values (7.33), on the

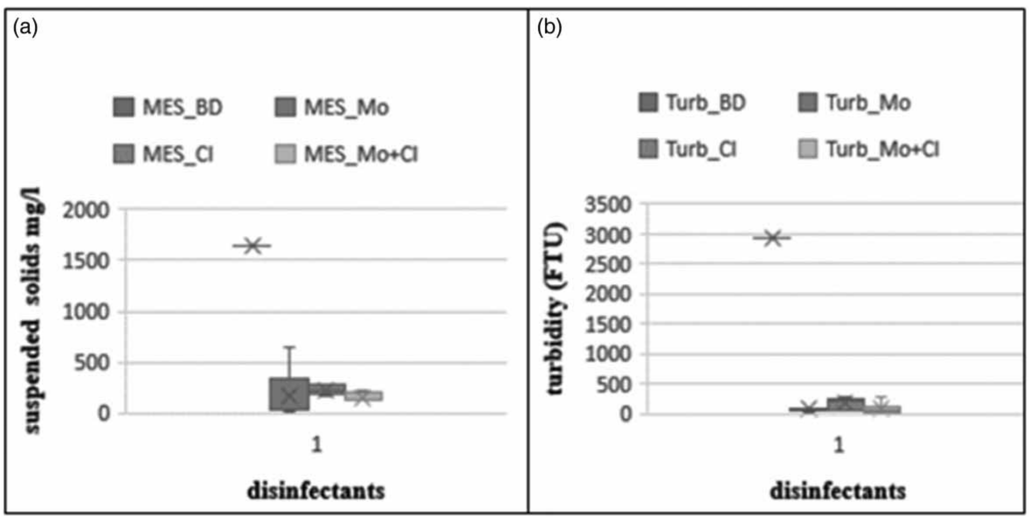

Figure 6 | Variation of suspended solids (a) and turbidity (b).

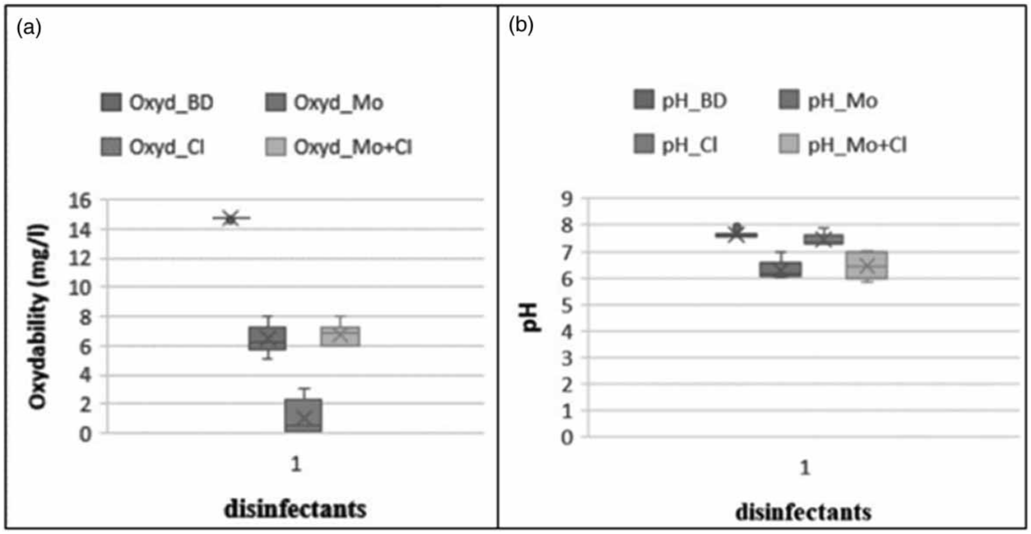

Figure 7 | Variation in oxidability (a) and pH (b).

other hand, samples treated with the other two disinfectants (*Moringa* and chlorine) have slightly acidic values of 6.05 and 5.86 (Figure 7(b)), respectively, for *Moringa* and *Moringa* associated with calcium hypochlorite.

Effectiveness of disinfectants on microorganisms and physicochemical quality

Efficiency yields vary depending on disinfectants and physicochemical and biological parameters. Table 1 shows the efficiency yields obtained with the different disinfectants at the C6 concentration. These analyses show that the yield obtained with *Ascaris* eggs ranges between 96% and 97%, while for *Ankylostoma* and *Trichuris* these yields vary between 98% and 100%. Only the *Moringa* equipped with chlorine yielded 100% yields for these two parasites. For the physicochemical parameters, the yields obtained for ammonia nitrogen, nitrate and orthophosphate with chlorine-treated samples are greater than 93%, while for samples treated with *Moringa* these yields remain less than 78%. For conductivity and turbidity, the highest yields obtained with the *Moringa* associated with chlorine are 93.4% and 99.72%, respectively. Calcium hypochlorite

Table 1 | Efficiency yields obtained with the different disinfectants R = [(initial value − residual value)/initial value] × 100

D	Co	Mo	Cl	Mo + Cl
Ascaris	3,500	120	102	94
	R	96.5	97	97.3
Ankylostoma	2,060	21	22	0
	R	98.98	98.93	100
Trichuris	1,500	2	7	0
	R	99.86	99.5	100
NH_4^+	16	11.4	0.9	1.33
	R	28.75	94.37	91.68
NO_3^-	151	32	10	19
	R	78.8	93.37	87.41
PO_4^{3-}	16	11.88	0.39	4.93
	R	25.75	97.56	69
Cond	6,805	1,929	797	443
	R	71	88	93.4
SS	1,645	640	180	220
	R	61	89	86.6
Turb	2,918	25	58	8
	R	99	98	99.72
Oxy	14.81	5.13	0	5.92
	R	65.36	100	60

Turb: turbidity; SS: suspended solids; NO_3^-: nitrate; NH_4^+: ammonia azote; PO_4^{3-}: orthophosphates; Cond: conductivity; Oxy: oxidability; Co: control; Mo: *Moringa*; Cl: calcium hypochlorite; Mo + Cl: *Moringa* associated with chlorine.

resulted in the highest yields on oxidable materials and suspended solids at 100% and 89%, respectively.

Pearson correlations

Significant and positive correlations were recorded at the 1% and 5% threshold (Table 2) between certain physicochemical parameters and the number of viable eggs counted. Before disinfection, the levels of ammonia nitrogen ($r = 0.95$), nitrate ($r = 0.93$), orthophosphate ($r = 0.94$), suspended solids ($r = 0.987$) and turbidity ($r = 0.92$) were positively and significantly correlated with the number of viable eggs counted before disinfection. Significant and negative correlations were also obtained between the levels of suspended solids ($r = 0.91$), turbidity ($r = 0.82$) and the number of viable eggs observed in the sample treated with Mo + Cl; as well, the pH ($r = 0.92$) is also positively and negatively correlated to the number of viable eggs obtained from the calcium chloride sample.

The levels of nitrates ($r = 0.984$) and suspended solids ($r = 0.96$) obtained after disinfection were positively and significantly correlated with the number of viable eggs counted in the control sample; conversely, suspended solids ($r = 0.96$) were significantly correlated with the number of viable eggs counted in the control sample. The levels of ammonia nitrogen ($r = 0.91$), nitrate ($r = 0.906$), suspended solids ($r = 0.89$) and turbidity ($r = 0.94$) measured after disinfection are significantly and positively correlated with the numbers of eggs counted in the sample treated with *Moringa*. Significant and positive correlations were also observed between nitrate levels ($r = 1.000$), suspended solids ($r = 0.94$) and turbidity ($r = 0.92$) and the number of viable eggs counted in the *Moringa*-treated sample associated with calcium hypochlorite.

DISCUSSION

The presence of nematode eggs at very high densities in control samples (3,500 eggs/L, 2,060 eggs/L and 1,500 eggs/L, respectively, for *Ascaris, Ankylostoma* and *Trichuris*) for treatment would be due to the fact that these eggs do not need to go through an intermediate host to ensure their development. A simple passage of nematode eggs into the outdoor environment subject to favourable environmental conditions is necessary for the nematode eggs to retain their viability. They can remain infectious in soil and water for years (Papajova *et al.* 2008).

For *Ascaris* eggs the most effective disinfectant is calcium hypochlorite associated *Moringa* with an efficacy rate of 97.3%, while *Moringa* is the least effective disinfectant (96%). None of the three disinfectant solutions used achieved a 100% efficacy rate, due to the high resistance of *Ascaris* eggs to the various disinfection processes used worldwide (Rosypal *et al.* 2007). This resistance is due to the presence of a cuticle composed of several layers (three to four depending on the gender). This cuticle overlay prevents the passage of certain substances such as acids and strong bases, oxidants, reducing agents and detergents (Wharton 1983). The permeability of the egg is limited to the passage of water, certain solvents and gases (Keffala *et al.* 2012). Indeed, *Ascaris* eggs represent the most resistant helminths to disinfection, and the complete inactivation of *Ascaris* eggs in waters may suggest that all other helminth

Table 2 | Table of correlations between the physicochemical variables measured before and after disinfection and the number of viable eggs counted in the control sample and the treated samples

V NOV	NH_3^+ (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)	MES (mg/L)	Turb (FTU)	pH	NH_3^+ AD (mg/l)	NO_3^- AD (mg/L)	PO_4^{3-} AD (mg/L)	SS AD (mg/L)	Turb AD (FTU)
Co	0.95*	0.93*	0.94*	0.987**	0.92*	0.285	0.7	0.984**	−0.721	−0.96**	0.66
Mo	0.33	0.31	−0.779	−0.907*	−0.91	0.313	0.91*	0.906*	−0.856	0.89*	0.94*
Ca	0.47	0.062	−0.606	−0.348	−0.26	−0.92*	0.43	0.980*	−0.92*	0.51	0.57
Mo + Cl	0.62	0.016	−0.455	−0.91*	−0.82*	0.235	0.39	1.000**	−0.601	0.94*	0.92*

*Significant correlations to 0.5; **Significant correlations at 0.01.

pH: hydrogen potential; Turb: turbidity; SS: suspended solids; NO^{3-}: nitrate; NH_4^+: ammonia azote; PO_4^{3-}: orthophosphates; NO_3^-AD: nitrate after disinfection; PO_4^{3-}AD: orthophosphates after disinfection; NH_3^+ AD: nitrogen ammoniacal after disinfection; Turb AD: turbidity after disinfection; SS AD: suspended solids after disinfection; NOV: number of viable eggs.

eggs present in the environment are inactivated (Paulsrud *et al.* 2004). The inactivation of *Ascaris* eggs requires significant action of the active ingredients of disinfectants to oxidize the three membranes that protect the genetic material of *Ascaris* eggs and allow DNA destruction or inactivation. Studies conducted by Massara *et al.* (2001) revealed that only one out of 16 disinfectants tested on *Ascaris* eggs had completely inhibited their segmentation. *Ascaris* eggs have a low negative surface load (Capizzi & Schwartzbrod 2001), which allows *Moringa* proteins to bind to the surface of the egg and facilitate their inactivation.

For the eggs of *Ankylostoma*, only calcium hypochlorite associated with *Moringa* achieved an efficacy rate of 100%; the rate of efficacy obtained with the other two disinfectants varies at around 98.9%. Similarly, for *Trichuris* eggs, *Moringa* combined with calcium hypochlorite is also the most effective disinfectant with a 100% efficacy rate; the efficacy rate obtained with the other two disinfectants (*Moringa* and chlorine) was greater than 99.5%. The hookworms and *Trichuris* eggs have a strong, rigid membrane that allows the cytoplasmic content and genetic material to withstand disinfection.

The high observed efficacy of *Moringa* associated with calcium hypochlorite compared to the other two disinfectants used, is believed to be due to the fact that we combined the two disinfectants (*Moringa* and chlorine) at equal proportions, and the combined action of active ingredients of these two disinfectants therefore promotes oxidation of the membranes of the eggs. The *Ascaris* eggs are the most resistant of the three parasites tested due to the triple membranes that protect the genetic material. Conversely, the eggs of *Ankylostoma* and *Trichuris* have a single membrane and are therefore more exposed than the eggs of *Ascaris*. During the study, *Trichuris* eggs showed low resistance compared to *Ankylostoma* eggs. The fragility of *Trichuris* eggs compared to the other two may be due to the presence of mucous plugs at their end, which may constitute fragility points or contact sites facilitating the penetration of disinfectant into the egg; in fact, mucous caps in some eggs may peel off when *Trichuris* eggs are exposed to high levels of disinfectant.

When the concentration of helminth eggs in water is high, several processes must be combined to reach the recommended limits (Keffala *et al.* 2012). The high efficacy of *Moringa* combined with calcium hypochlorite presents a good alternative for effective water treatment and, in fact, calcium hypochlorite has not allowed inactivation of all the eggs of helminths. Calcium hypochlorite, being a chemical, is not very advisable at high doses when treating water. *Moringa*, which is an organic disinfectant, has no adverse health effects and is used as a dietary supplement. The rational use of calcium hypochlorite associated with *Moringa* is a good alternative for optimal treatment of nematodes in water. *Moringa* does not produce dangerous by-products, and provides greater biodegradability. This effectiveness in adsorption of chemical particles by *Moringa* has already been demonstrated in reducing turbidity in water (Arantes *et al.* 2012) and pH adjustment (Amagloh & Benang 2009).

Several physicochemical parameters can influence the effectiveness of helminth treatment (Jiménez 2007). pH is a parameter that significantly influences the treatment of water from chlorinated products (Rodier 2009). In general, almost all of the pH values obtained from chlorine-treated samples tend towards basicity and these basic pH values obtained with chlorine will result in low production of hypochlorite acid which is the most chlorine element active on microorganisms. This will negatively influence the inactivation of microorganisms by chlorine (Rodier 2009). Conversely, samples treated with simple *Moringa* and those treated with *Moringa* associated with chlorine have slightly acidic pH values which allows a large production of hypochlorous acid, which could explain the high rates of yield for *Moringa* associated with chlorine. pH is a parameter that influences the treatment of helminths.

The values of nitrates, ammoniacal nitrogen and oxidability obtained from control samples before disinfection are higher than the standards prescribed by Rodier (2009). After application of disinfectants some of these values are significantly reduced but they remain present in all samples. Nitrates obtained after treatment are all above 9 mg/L, so ammoniacal nitrogen values obtained after disinfection are also greater than 1.1 mg/L. Apart from the chlorine-treated sample where the value of oxidability is 0 mg/L, the other oxidability values obtained after disinfection remain above 0.23 mg/L. Ammoniacal nitrogen is a parameter to be considered when treating water (Pecson *et al.* 2007). The significant and positive correlations observed between ammonia nitrogen, nitrates, orthophosphate, turbidity, suspended solids and counted viable eggs confirms the role played by organic matter during the process of disinfection. Organic matter can absorb disinfectant and make unstable the amount of disinfectant needed to inactivate microorganisms, while suspended solids and particles in water can be a barrier between the disinfectant and microorganisms, thus limiting the contact between the disinfectant and the target microorganisms.

The values of suspended solids (1,640 mg/L) and of turbidity (2,920 FTU) obtained in the control sample before disinfection are very high. These values drop considerably after applications of disinfectants, but remain higher than recommended values (2 FTU for turbidity and 50 mg/L for suspended solids) per USEPA (2004) for water reuse. The highest yield for suspended solids elimination was achieved with chlorine (89%); while for turbidity the highest yield was obtained with chlorine-associated *Moringa* (99.72%). Similar observations were made by Muyibi & Evison (1995) who achieved a reduction in turbidity of 92% and 95%, respectively, with *Moringa* extracts. According to Sengupta *et al.* (2012), *Moringa* is very effective in reducing turbidity and treating helminths, and this efficiency is all the greater when the water is less turbid. The presence of these microparticles after disinfection promote the protection of nematode eggs from the biocide action of disinfectants. Significant and negative correlations were observed between the suspended solids, turbidity and the number of viable eggs. The germicidal power of chlorine decreases sharply when water turbidity exceeds 5 NTU (Shimizu *et al.* 1997). The limited action observed between the different disinfectants used and the eggs could be explained by the persistence of certain micro-particles after disinfection. The WHO recommends an average turbidity of 1 NTU for water for treatment.

CONCLUSION

The purpose of this study was to evaluate the effectiveness of three disinfectant solutions (*Moringa*, calcium hypochloride, *Moringa*–chlorine) on the eggs of nematodes isolated from the floodwaters of the University City of Yaounde I University. This study found that three species of parasites (*A. lumbricoides*, *Ankylostoma* sp. and *Trichuris* sp.) were identified in samples used for treatment. These species have very variable densities, and different sensitivities when they are subjected to disinfection treatment. Of the three disinfectant solutions used, *Moringa* combined with chlorine is the most effective disinfectant compared to the other two disinfectants used. *Ascaris* eggs showed greater resistance compared to the eggs of *Ankylostoma* sp. and *Trichuris*. In addition, certain abiotic parameters can influence the process of water treatment, either by consuming part of the disinfectant or by creating a barrier limiting contact between microorganisms and disinfectants. The use of disinfectants for water treatment must take into account the parasitological composition of the water to be treated, thus, the more parasites there are, the higher the concentration of the disinfectant must be. In view of the harmful effects of chemicals, it would be more sensible to use disinfectants based on plant extracts in wastewater treatment.

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