Alexandria Journal of Veterinary Sciences 2013, 39: 82-90
ISSN 110-2047
www.alexjvs.com

Assessment Efficiency of Some Chemical Disinfectants Commonly Used Against Coccidia in poultry Farms

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Key words:
Chemical
Disinfectant
Coccidia
Poultry farm

ABSTRACT:

Efficiency of four commercial disinfectants including ammonium hydroxide, phenol, Eco.Bio (combination of QACs and gluteraldehyde) and Zixvirox (combination of peracetic acid and hydrogen peroxide) was evaluated against unsporulated *E. tenella* oocysts which were obtained from naturally infected birds with ceccal coccidiosis then propagated in broiler chicks reared on wire cage and fed anticoccidial free starter ration from day one of rearing till the end of experiment. The oocysts were exposed to the disinfectants at different concentrations and different contact times. In addition, the effect of some environmental factors including temperature, pH and presence of organic matter on the efficiency of these disinfectants were also studied. Based on obtained results of the current study, it was observed that the most effective disinfectants against unsporulated *E. tenella* oocysts were ammonium hydroxide 5% and 10% and Phenol 10%. While Eco. Bio and Zixvirox, were less effective. Also, the results proved that the inhibitory activity of the tested disinfectants was greatly increased by increasing their concentrations or the contact time. Moreover, rising temperature increased the efficacy of all tested disinfectants except Zixvirox. Addition of organic matter in the form of (10% chicken faeces) reduced the efficacy of both Zixvirox and Eco.Bio disinfectants by 52.9% and 47%, respectively. While Ammonium hydroxide and Phenol disinfectants were not affected by addition of organic matter. Finally, the highest efficacies of both Ammonium hydroxide and Eco.Bio disinfectants were attained at alkaline pH, while for Phenol and Zixvirox were active at acidic pH.

1- INTRODUCTION:

Broiler industry becomes one of the most important industries around the world. As it share in production of 7.6 billion chicken annually (Allen and Fetterer, 2002) that represents a considerable percent of consumed meat all over the world. Unfortunately, development of this industry can be seriously restricted under both farm yard and modern conditions (Conway and McKenzie, 2007) due to a disease called avian coccidiosis that is the most widely reported disease of poultry in the world (Dalloul and Lillehoj, 2006). Coccidiosis is an intestinal infection caused by the *Apicomplexan* protozoan belonging to the subclass *Coccidia*, family *Eimeriidae* and genus *Eimeria* (Finlay et al., 1993;

Lillehoj&Lillehoj, 2000).The global cost due to coccidiosis probably exceeds £500 M per annum (Shirley, et al., 2007). Current control of the *Eimeria* species is based primarily on the use of medication by anticoccidial drugs (Shirley, et al., 2007). An alternative to the chemotherapeutic treatment of coccidiosis would be early disinfection of the poultry buildings especially under the conditions of intensive livestock housing. Williams, (1997) mentioned that ammonia, methyl bromide, carbon disulphide and phenols were found to have oocysticidal activity. Evaluation of the efficiency of commercial disinfectants against *E. tenella* under different environmental factors was done by many authors including LongXian, et al., (1999), Daugschies, et al., (2002), Desouky and El-Midany (2003), Oliveira, et al., (2004), Shalaby, et al., (2004) and Junior, et al., (2007). So, this study was carried out as a trial for finding out the most effective commercial chemical disinfectant against *E. tenella* oocysts and the most suitable conditions for its application.

2- MATERIALS AND METHODS:

2.1. *E. tenella* oocysts:

It was obtained by collecting number of caeci of infected birds with caecal coccidiosis that processed to extract the *E. tenella* oocysts. The harvested oocysts from caecal tissue were purified in order to reduce the effect of organic matter to negligible value by using flotation technique then the number of oocysts was adjusted to 25000 oocysts per ml using MacMaster slide (Nematollahi et al., 2008).

2.2. selected chemical disinfectants:

2.2.1. Phenol:

It was obtained from Mega Pharma Company for veterinary products and disinfectants and it was used at concentrations of 5%, 10% and 20%.

2.2.2. Eco Bio®: (Eco Bio UK limited, cherry tree house, London, England):

Each liter of Eco Bio composed of (Alkyl dimethyle benzyl ammonium chloride 50 g + alkyl dimethyl ethyl ammonium chloride 50 g + Isopropanol 50 g + gluteraldehyde 120 g). It was used at concentrations of 5%, 10% and 20%.

2.2.3. Ammonium hydroxide 33%:

It was obtained from El- Nasr pharmaceutical Chemicals Co. and it was used at concentrations of 2.5%, 5%, and 10%.

2.2.4. Zixvirox: (United BioMed.):

It is a combination of peracetic acid and hydrogen peroxide and it was used at concentrations of 2%, 4% and 6%.

2.3. In vitro evaluation of the tested disinfectants against unsporulated *E. tenella* oocysts under different conditions:

2.3.1. Effect of concentration and contact time:

1 ml of oocysts solution was incubated with each concentration of the different tested disinfectants at time contact of 10, 30, 60, 90 minutes and 24 hours at temperature of 25 ºC. At the end of each contact time, the disinfected oocysts were washed by distilled water 3 times to remove the effect of the disinfectant then 1 ml of potassium dichromate solution (2.5%) was added to the washed oocysts in each tube then the tubes were kept in air at room temperature for sporulation. Also, control tubes containing non exposed oocysts to any disinfectant solutions were prepared.

2.3.2. Effect of temperature:

Tested disinfectants (ammonium hydroxide 10%, phenol 10%, Zixvirox 4% and Eco Bio 10%) were added to 1 ml of oocysts solution at different temperature of 4, 10, 20, 30 and 40 ºC at fixed contact time of 15 minutes. After the end of the experiment, the disinfected oocysts undergo the same previously mentioned procedures.

2.3.3. Effect of pH:

The tested disinfectants at different concentrations (ammonium hydroxide

10%, phenol 10%, Zixvirox 4% and Eco Bio 10%) were added to 1 ml of oocysts solution at different pH values; 2, 5, 7, 9 and 11 for (phenol 10% and Eco Bio 10%), pH of 2, 5, 7, 9 and 11.4 for Zixvirox and pH of 8.6, 9.4, 10, 11 and 12 for ammonium hydroxide 10%. At temperature of 25 ºC and fixed time contact of 15 minutes. After the end of the experiment, the disinfected oocysts undergo the same previously mentioned procedures.

2.3.4. Effect of organic matter:

Stock solution containing organic matter (chicken faeces 10%) was prepared by adding 10 g of chicken faeces to 100 ml of distilled water. Tested *E. tenella* oocysts were added to the solution of organic matter, and then the number of oocysts was adjusted to 25000 oocysts per ml. Another oocysts solution was prepared using distilled water only and each ml contained 25000 oocysts. Tested disinfectants (ammonium hydroxide 10%, phenol 10%, Zixvirox 4% and Eco Bio 10%) were added to 1 ml of oocysts solution in presence and absence of organic matter for 15 minutes at a temperature of 25 ºC. Control tube containing oocysts with distilled water were also prepared. After the end of the experiment, the disinfected oocysts undergo the same previously mentioned procedures.

2.2.5. The efficacy of the tested disinfectants was evaluated by obtaining their Inhibitory Activity (IA %) (sporulation inhibition% of the oocysts) as follow: after at least 7 days of each experiment, the number of sporulated oocysts in each tube were counted using MacMaster slide to get the percentage of sporulation, this percentage was subtracted from sporulation % of the control tube to get inhibitory activity of the tested disinfectant.

IA = {(sporulation % of control - sporulation % of disinfected oocysts) × 100}/ sporulation % of control

3- RESULTS & DISCUSSION

Table (1): Effect of concentration and contact time on efficacy of tested disinfectants:

C. T.	Ammonium hydroxide						Phenol						Zixvirox						Eco Bio					
	2.5%		5%		10%		5%		10%		20%		2%		4%		6%		5%		10%		20%	
	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %
10 min.	45	47	25	70	18	78.8	40	33.1	33.3	44.3	27.2	54.5	57.1	4.5	52.9	11.5	44.2	26	51.5	13.8	45.5	23.9	42.8	28.4
30 min.	30.5	64.1	19.6	76.9	16.6	80.4	40	33.1	33.3	44.3	20	66.5	56.6	5.3	52.4	12.3	42.8	28.4	49.3	17.5	41.3	30.9	39.1	34.6
60 min.	28	67	3.5	95.8	3.2	96.2	40	33.1	28.5	52.3	11.1	81.4	46	23	46.5	22.2	36.5	38.9	48.5	18.8	33.3	44.3	31.5	47.3
90 min.	23	72.9	3	96.4	2.5	97	37.5	37.2	25	58.1	10	83.2	45.4	24	45	24.7	33.9	43.3	41.5	30.6	28.5	52.3	24.6	58.8
24 hrs.	5	94.1	0.5	99.4	0	100	11.1	81.4	0	100	0	100	41.3	30.9	41.6	30.4	30	49.8	30.8	48.4	27.2	54.5	14.2	76.2
control	85	-----	85	-----	85	-----	59.8	-----	59.8	-----	59.8	-----	59.8	-----	59.8	-----	59.8	-----	59.8	-----	59.8	-----	59.8	-----

SP: Sporulation. IA: Inhibitory Activity.

Table (2): Effect of presence of organic matter on the efficacy of tested disinfectants:

Disinfectant	SP% in absence of organic matter	IA % in absence of organic matter	SP %in presence of organic matter	IA % in presence of organic matter
Amm. hyd. 10%	2.7	95.8	4	93.8
Phenol 10%	37.5	42.1	33.3	48.6
Zixvirox 4%	53.6	17.2	59.5	8.1
Eco.Bio 10%	53.9	16.8	59	8.9
Control	64.8	-------	64.8	-------

SP: Sporulation. IA: Inhibitory Activity.

Table (3): Effect of temperature on the efficacy of tested disinfectants:

T (ºC)	Ammonium hydroxide 10%		Phenol 10%		Zixvirox 4%		Eco.Bio 10%	
	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %
4	17	77.2	45.4	40	47.8	36.8	45.2	40.2
10	15.3	79.7	38.8	48.7	45.6	39.7	44.8	40.8
20	15.3	79.7	28.5	62.3	47.1	37.7	39.3	48
30	12.8	83	20	73.5	46.3	38.8	39.3	48
40	0	100	25	66.9	48	36.5	38.4	49.2
Control	75.7	-----	75.7	-----	75.7	-----	75.7	-----

SP: Sporulation. IA: Inhibitory Activity.

Table (4): Effect of pH on the efficacy of tested disinfectant:

pH	Ammonium hydroxide10%		Phenol 10%		Zixvirox 4%		Eco.Bio 10%	
	SP %	IA %	SP %	IA %	SP %	IA %	SP %	IA %
2	-----	-----	44	41.7	53.9	28.6	61.8	18.1
5	-----	-----	55.5	26.4	44.1	41.5	56	25.8
7	-----	-----	60	20.5	55.1	27	51.8	31.3
8.6	10	86.7	-----	-----	-----	-----	-----	-----
9	9	88	60	20.5	61.5	18.5	51.7	31.5
10	6.5	91.3	-----	-----	-----	-----	-----	-----
11	3.3	95.6	75	0.7	-----	-----	40	47
11.4	-----	-----	-----	-----	66.6	11.7	-----	-----
12	3	96	-----	-----	-----	-----	-----	-----
Control	75.5	-----	75.5	-----	75.5	-----	75.5	-----

SP: Sporulation. IA: Inhibitory Activity.

Poultry coccidiosis represents a real threat for poultry industry worldwide as well as in Egypt, as it causes great economic losses. Unfortunately, the causative agents of this disease "*Eimeria* oocysts" are highly resistant to most known chemical disinfectants. McDonnell and Russell (1999) classified the oocysts as the most resistant etiological agents to the disinfectants, after the Prions. According to Daugschies et al., (2002) an inhibitory activity (IA) of at least 95% was required for certification of sufficient disinfecting efficacy by the German

Veterinary Society. The effect of concentration and contact time (CT) on efficacy of tested chemical disinfectants against unsporulated *E. tenella* oocysts was tabulated in Table (1). Firstly, it was observed that ammonium hydroxide was effective at concentrations of 5% at CT of 60 and 90 minutes and 24 hours where the IA reached 95.8%, 96.4% and 99.4%, respectively. The obtained result was in agreement with that obtained by Horton-smith, et al., (1940) who found that 5% ammonia solution destroyed oocysts of *E. tenella* in 24 hours and Zahran, (1979) who found that 5% ammonium hydroxide destroyed *Eimeria* oocysts causing rabbit coccidiosis in 24 hours. Also, it was recorded that 10% concentration showed IA of 96.2%, 97% and 100% at 60 and 90 minutes and 24 hours, respectively that was similar to that obtained by Desouky and El-Midany (2003) who recorded 100% IA for ammonium hydroxide (33%) at concentration of 10% after CT 24 hours. on contrary, it was differed from that recorded by LongXian, et al., (1999) who found that 10% ammonia water for 30 minutes inhibited the development of the unsporulated oocysts in chicken feces. Secondly, the obtained results in the current study clarified that phenol was highly effective at concentration of 10% for 24 hours CT with IA reached 100%. This result was nearly similar to that obtained by Williams (1997) who found that phenol 41.6% showed similar efficacies in low concentrations. On contrary, it disagreed with Oliveira, et al., (2004) who found that phenol 10.5% + cresol 10.5% at exposure time of 30 minutes had 7.7% IA. At the same time, 5% phenol showed 81.4% IA after 24 hours CT that was lower than that recorded by Desouky and El-Midany (2003) who found that commercial phenol (5%) showed 94.3% IA after the same CT.

Data tabulated in Table (1) also clarified that the effect of Zixvirox disinfectant on inhibition of sporulation of *E. tenella* oocysts was disappointing, as concentration of 2% (the recommended concentration by producer to kill coccidia oocysts) recorded 4.5% IA after 10 minutes CT. Moreover, by increasing the contact time to 24 hours, its efficacy was still limited as it reached 30.9% only. Furthermore, by increasing the concentration of Zixvirox to 6%, it was noticed that the recorded IA was 49.8% after 24 hrs CT that was still non sufficient to classify the product as coccidicidal disinfectant. This obtained result was in harmony with that obtained by Fayer et al., (1997) who found that high concentrations of hydrogen peroxide and peracetic acid were of limited activity against coccidian oocysts and Shalaby et al., (2004) who found that 2% (hydrogen peroxide 50% and dihydroxybenzol 100 ppm) solution had 53.6% IA against *E. tenella* oocysts at 24 hours CT. On contrary, the obtained result of Zixvirox disagreed with Queiroz de Souza et al., (2012) who stated that microorganisms of the subclass *Coccidia* were inactivated by hydrogen peroxide 6% and 7.5%,

Concerning, Eco.Bio disinfectant, highest IA 76.2% was obtained at 20% concentration at 24 hrs CT that was unexpected. This result may be due to the synergistic effect of gluteraldehyde and QAC combination as it was well known that QACs were lipophilic compounds so; it may act on the oocysts wall allowing gluteraldehyde (hydrophilic compound) to act on the content of the oocysts. Moreover, it was noticed that Eco.Bio recorded increased IA by increasing concentration and/or CT that agreed with Bessems (1998) who found that at a constant test concentration, the rate of killing was increased with an increase in time contact for membrane-active disinfectant including QAC. On contrary, these results disagreed with Zahran (1979) who tested two commercial quaternary ammonium disinfectants against unsporulated *Eimeria* spp. causing rabbit coccidiosis, and found that 1%, 3% and 5% of both products failed to kill the oocysts.

The effect of presence of organic matter as an expected factor during the field application of the tested disinfectants was illustrated in Table (2). It was observed that phenol was the lowest disinfectants affected by presence of organic matter followed by ammonium hydroxide as the inhibitory activity of ammonium hydroxide 10% was reduced only by 2 % due to the presence of 10% chicken faeces as a source of organic matter. While Phenol 10% was not affected completely by presence of organic matter. Moreover, its efficacy was increased by 6.5% in presence of organic matter. This result agreed with Berchieri and Barrow (1996); Amass, et al., (2000); Kennedy, et al., (2000); Quinn and Markey (2001) and BCCDC Laboratory Services (2003) while disagreed with Morishita and Gordon (2002) and Watkinson (2008) who mentioned that organic matter slightly inhibited the effectiveness of phenols**.** On the other side, Zixvirox and Eco.Bio disinfectants were greatly affected by the presence of 10 % chicken faeces as a source of organic matter as their efficacy was reduced nearby 50 % for each one. Concerning Zixvirox 4% solution, it was found that the inhibitory activity in absence of organic matter was 17.2% that was reduced to 8.1% in presence of 10 % chicken faeces that meant that its efficacy was reduced by 52.9%. This result agreed with Amass et al., (2001) and Block, (2002) while it disagreed with Lensing and Oei, (1984); Malchesky, (1993); Jeffrey (1995); Kitis (2004) and Rutala and Weber (2004). Concerning, Eco. Bio 10% disinfectant solution, it was found that its efficacy was reduced by 47% in presence of 10% chicken faeces where IA was reduced from 16.8% to 8.9 %. This result was in agreement with Berchieri and Barrow (1996); Rodgers et al., (2001); Dvorak (2005) and Payne, et al., (2005). On contrary, it disagreed with Martinez et al., (1999); National Seafood HACCP Alliance (2000) and Duong (2005).

Generally, most disinfectants worked best at temperatures above 68^{o}F however, elevated temperatures may accelerate evaporation of a disinfectant that could reduce contact time and could decrease efficacy. Also, colder temperatures might reduce the efficacy of some disinfectant products. In addition, the activity of alkali compounds was slow but can be increased by rising the temperature (Dvorak, 2005). The effect of different degrees of temperature on tested disinfectants against unsporulated *E. tenella* oocysts was summarized in Table (3). It was observed that there was an increased efficacy of all tested disinfectants by increasing the temperature except Zixvirox 4%, as increased temperature seemed to have no effect on its efficacy against *E. tenella* oocysts. 100% inhibition of oocysts sporulation was reached with disinfection by ammonium hydroxide 10% at 15 minutes CT and 40 ^{0}C. It is worthy to know that the same result was obtained after exposure of the oocysts to ammonium hydroxide 10% solution for 24 hrs when the temperature was 25^{0}C so using of ammonium hydroxide 10 % solution would reduce contact time from 24 hrs to 15 minutes by elevating temperature to 40^{0}C. Data illustrated in the same table also revealed that increased temperature greatly enhanced the efficacy of phenol 10% solution where the IA rose from 40% at 4 ^{0}C to be 73.5% at 30 ^{0}C. This result was in agreement with Russell, (1999) and Goddard and McCue, (2002*).* Also, Eco.Bio10% recorded increased efficacy by rising of the temperature but by lower degree than ammonium hydroxide and phenol; as its IA was 40.2% at 4^{0}C and reached 49.2% at 40 ^{0}C. This result was agreed with Langsrud and Sundhiem (1997).

Effect of pH degree on the efficacy of tested chemical disinfectant against unsporulated *E. tenella* oocysts was shown in Table (4) where it revealed that alkaline pH increased the efficacy of both ammonium hydroxide 10% and

Eco.Bio 10% disinfectants. For ammonium hydroxide the highest efficacy was obtained at pH 12 where IA reached 96%; nearly the same result was obtained at pH 11 (95.6% IA). While highest efficacy of Eco.Bio (47% IA) was reached at pH 11. This results agreed with Dvorak (2005) and Watkinson (2008). On the other hand, acidic pH was found to activate both Zixvirox and phenol disinfectants; where the highest efficacies for phenol 10% and virox4% were obtained at pH degree of 2 and 5, respectively. This result was agreed with Baldry and French, 1989; Sanchez-Ruiz et al., 1995; Goddard and McCue (2002); Gehr, et al., (2002) and Watkinson (2008). In conclusion: the most effective disinfectants against *E. tenella* oocysts were ammonium hydroxide 5% and 10% and phenol 10% at contact time of 24 hrs. Also, increasing temperature to 40 ^{0}C reduced the CT with ammonium hydroxide 10% to 15 mint. only. In addition, alkaline pH should be considered during application of ammonium hydroxide while acidic one should be maintained during disinfection by phenolic disinfectants. Moreover, organic matters presence had no effect on efficacies of both disinfectants. At the end, this study recommends the use of phenol 10% for 24 hrs. CT to eradicate the oocysts rather than the usage of ammonium hydroxide as it is irritating, suffocating and corrosive product that has deleterious effect on general health and also lower the expected life time of the poultry house and its utensils and equipment.

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