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DISINFECTION OF EGGS CONTAMINATED WITH SOME FUNGI AND MOULDS

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It is known that in the epizootology of avian aspergillosis, the role of incubator is essential where the high humidity and optimal temperature create prerequisites for development of fungi and molds, introduced with contaminated eggs (Enev et al., 2005). On the other side, molds could penetrate through the egg shell during storage in storerooms, thus deteriorating their quality and making them inappropriate for consumption.(Pavlov et al., 2006). With this matter there is a problem about biosecurity program, good hygien with an appropriate detergents and disinfectants.(Deeks, 2005, Baychev and Karadjov . 2006;. Luc, 2007;. Madec , 2007;. Best, 2007;). According Luc .(2002) the stabilized combination of hydrogen peroxide and organic acids clean and sanitise the water, destroying the microorganisms like salmonellae, E.coli, clostridia, Pseudomonas and streptococcal bacteria.. Using hydrogene peroxide – H2O2- for desinfection , Higgins et al.(2005). found out that this method is effective against Salmonella spp., and there was no detrimental effects on hatchability of broiler breeder eggs. The data of Froning .(2006). indicated that combining heat at 54^{o}C and ultrasonic waves provided a reduction of Salmonella enteritidis on the egg's shell.). Chlorin is effective against both enveloped and nonenveloped viruses, against fungi, bacteria and algae (Russell and Keener, 2007). A Disinfection with Lugol's solution, chlorxedine, ethanol and quarternary ammonium solutions faile to achive complete decontamination of the egg's shell experimentally contaminated with Salmonella enteritidus (Himathongkham et al., 1999). Almost identical results have been obtained with a tendency of better stimulation of hatching by disinfection with ozon compared to disinfection with formaldehyd of hatching eggs for broiler chicken (Chmelnicna, 2000). According Davies and Breslin (2003) the contamination in egg-packing plants may be a significant contributory factor to external contamination of shell eggs, and improved methods of cleaning and disinfecting egg-handling equipment are required.

These circumstances above raise the question for an optimization of choice of disinfectants and methods for their application for decontamination of egg's shells, contaminated with some moulds.

Experimental design. In the experiment, eggs obtained from layers, free of aspergillosis, were used. The eggs were infected via dipping with a suspension of spores from Aspergillus spp.- flavus, niger,fumigatus (30 eggs), Mucor spp.,- mucedo, spherosporum (30 eggs), Penicillium spp.-notatum, aromaticum (30 eggs), E.coli 078, E.coli 026, Staphilococus aureus. The spore material was dosed to 50 000 spores respectively bavterial cells per cm^2 of the egg shell.

Infected eggs were treated with the following disinfectants:

1. Iodination of eggs via immersion for 1 (one) minute in a solution of 80 parts boiled water + 20 parts 5% tincture of iodine at a temperature of 20^{o} C.
2. Fumigation with formaldehyde, obtained from 60 mL formalin, 30 mL water and 48 g potassium permanganate at a temperature of 37^{o} C, air humidity 80% and exposure time 30 minutes.
3. Immersion of eggs in 1% solution of beta-propiolactone for 1 (one) minute at a temperature of 20^{o} C.

4. Immersion of eggs in 3 % copper sulphate solution ($CuSO_4$) (pH 4,0) added with 2 % hydrogen peroxide solution for 30 minutes at 20° C.
5. Immersion of eggs in 1,25% sodium sulphite (Na_2SO_3) solution (pH 9.5) for 30 min at 20° C.
6. Immersion of eggs in 1.25% sodium sulphide (Na_2S) solution (pH 11.0) for 30 minutes at 20° C.
7. Immersion of eggs in 1.25% sodium persulphate ($Na_2S_2O_8$) solution (pH 4.0) for 30 min at 20° C.
8. Immersion of eggs in 1.25% sodium thiosulphate ($Na_2S_2O_3$) solution (pH 7,0) for 30 min at 20° C.

After the treatments, saline washings were obtained from egg's shell surfaces and inoculation in Saburaud's nutrient medium were performed for fungi and simple agar for bacteria.. The aftereffect of iodine was neutralized with 0.1% sodium thiosulphate solution, this of formalin – with 0.1% ammonium base, that of copper sulphate – with 0.1% sodium bicarbonate. The cultures were maintained at 25° C and the growth (appearance of colonies, presence of mycelium) was monitored by post inoculation days 3 and 7 for fungi. The cultures for bacteria . were maintained at 37° C and the growth (appearance of colonies) was monitored by post inoculation days 1 and 2 .

The safety of disinfection upon the hatchability of eggs was tested via incubation of 20 eggs, treated with either iodine, formalin or beta-propiolactone. Eggs, non-treated with disinfectants, served as controls.

The obtained results (table 1) showed that the best effect against all moulds had the combination formaldehyde with iodination and formaldehyde with beta-propiolactone. Iodination and formaldehyde treatment (60 g) had the most consistent effect. These data support the statement of Luc (2007).that a more efficient removing of microorganisms is gained by a better sanitation process. At the same time the Sodium thiosulphate, sodium sulphite and sodium persulphate did not exhibit antifungal activity.

Table 1 *– Antifungal activity of different chemicals on moulds on eggs' surface*

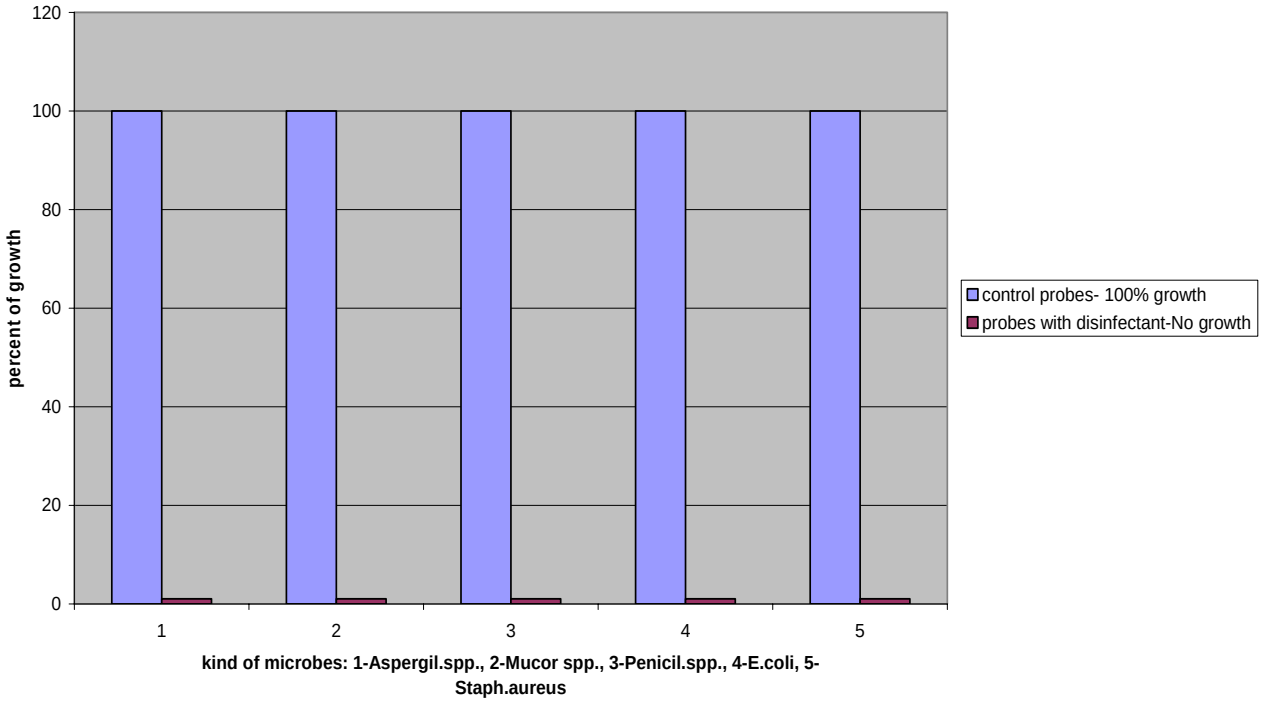
Chemicals	Moulds		
	Aspergillus spp.	Mucor spp.	Penicillium spp.
1. Jodine	(-)	(-)	(-)
2. Formaldehyde	(-)	(-)	(-)
3. beta-propiolacton	(-)	(-)	(-)
4. Sodium sulphite	(+)	(+)	(+)
5. Sodium sulphide	(-)	(-)	(-)
6. Sodium persulphate	(+)	(+)	(+)
7. Sodium thiosulfate	(+)	(+)	(+)
7. Sodium thiosulfate	(+)	(+)	(+)
8. Control growth	(+)	(+)	(+)
Note: (+) – growth, (-) – absence of growth			

The table 2 and the figure 1 bring out that a combination of 3% cupric sulphate and 2% of hydrogen peroxide inactivate fungi and bacteria as well. This result corresponds with the data of Luc (2002) showing that the stabilized combination of hydrogen peroxide and organic acids destroying the microorganisms like salmonellae, E.coli, clostridia, Pseudomonas and streptococcal bacteria.

Disinfected eggs showed a hatchability, higher by 10-30% than untreated eggs. Probably, it was due to elimination of microbial flora on the surface of egg's shell. The other researchers (Higgins et al, .2005). found out also that the using hydrogene peroxide for desinfection of carcasses, table eggs and fertile eggs didn't have any detrimental effects on hatchability of broiler breeder eggs .

Table 2 *– Desinfection effect of a combination of cupric sulphate 3% and hydrogen peroxide 2% on eggs contaminated with some fungi and bacteria at the exposition of 30 minutes(12.12.06)*			
Fungus&bacteria	Number of eggs	Results of trial	Control
Aspergillus flavus	6	(-)	(+)
Aspergillus fumigatus	6	(-)	(+)
Aspergillus niger	6	(-)	(+)
Mucor mucedo	6	(-)	(+)
E.coli 078	6	(-)	(+)
E.coli 026	6	(-)	(+)
Staph.aureus	6	(-)	(+)
Remark: (+) – growth; (-) – no growth			

Fig.1-Disinfection effect of a combination of cupric sulphate 3% and hydrogen peroxide 2% on egg's shell contaminated with some fungi and bacteria



In conclusion, it should be stated that the surface of egg shells was efficiently decontaminated by used chemicals. They are inexpensive, available, easily applicable and safe for work, and could be used for prevention and control of aspergillosis in poultry breeding (poultry farms, hatcheries, stores, litter).

REFERENCES

1. Baychev J., Karadjov S. (2006). Bird flu. Panta-Neo, Sofia., pp.104-144.
2. Best P. (2007).Risk assessors probe transport hygiene. Pig Int., v.37, # 4 , pp.6-7.
3. Chmelnicna M.(2000). Hatchability of broilers after disinfection hatching eggs with ozone. Int.sci. conference "Bioclimatology and environment", Kosice, Slovak Republic,13-14 September.
4. Davies R.H., M. Breslin (2003). Investigation of Salmonella contamination and disinfection in farm egg-packing plants. J.appl. microbial., v.94, # 2, pp.191-196.
5. Deeks A.(2005). Biosecurity doesn't cost. It pays. Poultry Int., v.44, #12, pp.14-16.
6. Enev I., Kostov K., Ivanova D., Badarova P. (2005). Aspergillosis in birds. In " Infectiouse diseases of animals.". Matcom, Sofia, pp. 252.
7. Froning G.(2006). Thermoultrasonication proposed for pasteurizing shell eggs. Poultry Int., v.45, #1, p. 24.

8. Higgins et al.(2005). Application f ionized reactive oxygen species for desinfection of carcasses, table eggs and fertile eggs. Journal of Applied Poultry Research, 14 : 716-720.
9. Himathongkham S., H.Riemann, R. Ernst (1999). Efficacy of disinfection of shell eggs externally contaminated with Salmonella enteritidis implications for testing. Int.J. Food microbial., v.49, # 3, pp.161-167.
10. Luc L.(2002). Clean water lines. Poultry Int., v. 41, #2, pp.34-36 .
11. Luc W.(2007). Good sanitation needs a detergent. Pig Int.,v.37, #4, pp.12-13.
12. Madec F., (2007).Keep out those pathogens. Pig Int., v.37, # 4 , pp.8-9.
13. Pavlov A., I. Vushin, H. Daskalov (2006). Hygien, technology and veterinary – sanitary control of meat, fish and eggs. Kota, Stara Zagora, pp. 156- 159.
14. Russell S., Keener K.(2007). Chlorine – misunderstood pathogen reduction tool. Poultry